# TRACE ELEMENT ANALYSIS OF SERUM FROM BONE MARROW TRANSPLANT PATIENTS USING NEUTRON ACTIVATION ANALYSIS

BY

EDUARDO MUSSNICH BARRETO

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1984

ACKNOWLEDGMENTS

The support and individual attention received from Dr. Genevieve Roessler, supervisory committee chairman, of the Department of Nuclear Engineering Sciences, and Dr. W. Emmett Bolch, academic advisor and supervisory committee cochairman, of the Department of Environmental Engineering Sciences, are gratefully acknowledged.

The faculty and administrative staff of the Department of Nuclear Engineering Sciences was extremely supportive during my research period at that department. My special thanks to the staff of the University of Florida Training Reactor for their time and effort in the irradiation of the samples.

The technical support available from Dr. J. Cerda, Dr. T. Baumgartner and nurses at the Bone Marrow Transplant Unit of Shands Hospital is also gratefully acknowledged.

I particularly thank my wife, Carmen, for her support and help with the drawings and final preparation of this manuscript.

I also thank the Brazilian Commission for Nuclear Energy (Comissao Nacional de Energia Nuclear) for making possible my graduate education.

TABLE OF CONTENTS


PAGE

ACKNOWLEDGMENTS . . . . . ii

ABSTRACT . . . . . v

CHAPTER

I. INTRODUCTION . . . . . 1

Object of This Study . . . . . 1
The Disease and the Transplant . . . . . 5
Nutritional Aspects of the Bone Marrow Transplant . . . . . 9
Trace Elements in Nutrition . . . . . 9
Bromine . . . . . 12
Cobalt . . . . . 12
Copper . . . . . 13
Iron . . . . . 14
Selenium . . . . . 14
Zinc . . . . . 15
Trace Element Studies for Serum . . . . . 15
Bromine . . . . . 17
Cobalt . . . . . 17
Copper . . . . . 17
Iron . . . . . 17
Selenium . . . . . 17
Zinc . . . . . 18
Quantitative Analysis through Neutron Activation 19

II. METHODS--SPECIMEN COLLECTION . . . . . 22

Sampling . . . . . 22
Sampling Technique . . . . . 24
Syringes . . . . . 24
Needles . . . . . 25
Sample Preparation . . . . . 28
Protocol Description . . . . . 30

III. METHODS--SERUM ANALYSIS . . . . . 35

Irradiations . . . . . 35
Selection of Optimal Procedure . . . . . 35

Irradiation Scheme . . . . . 38
Resin Tests . . . . . 39
Contamination Assessment . . . . . 41
Primary Standards . . . . . 42
HAP . . . . . 45
Post Irradiation Procedures . . . . . 49
Testing of Alternative Procedures . . . . . 50
Radiation Exposure Levels . . . . . 54

IV. RESULTS AND DISCUSSION . . . . . 58

Results . . . . . 58
Discussion . . . . . 80

V. CONCLUSIONS . . . . . 91

APPENDIX

A. THE COMPARATOR METHOD APPLIED TO SERUM ANALYSIS . . 93

B. PREPARATION OF HAP FOR SODIUM REMOVAL FROM SERUM SAMPLES . . . . . 101

C. PRECISION AND ACCURACY OF CONCENTRATION DETERMINATIONS . . . . . 106

LIST OF REFERENCES . . . . . 108

BIOGRAPHICAL SKETCH . . . . . 112

Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

# TRACE ELEMENT ANALYSIS OF SERUM FROM BONE MARROW TRANSPLANT PATIENTS USING NEUTRON ACTIVATION ANALYSIS

By

Eduardo Mussnich Barreto

August 1984

Chairman: Genevieve Roessler, Ph.D.
Cochairman: W. Emmett Bolch, Jr., Ph.D.
Major Department: Environmental Engineering Sciences

Simultaneous analysis of the 7 clinically significant elements Cu, Br, Zn, Co, Se, K and Fe in blood serum was developed using multielement neutron activation analysis (NAA) at the University of Florida Training Reactor (UFTR).

The use of the comparator method eliminated 2 limitations associated with the UFTR: relatively low thermal flux ($1*10^{12}$ neutrons/$cm^2$s) and interrupted operation during night hours.

Hydroxy antimony pentoxide (HAP) resin was successfully employed in the removal of the sample Na (one of the most

serious limitations of NAA with regard to samples with a biological matrix) in contrast to recent discouraging literature reports. Tests confirmed Se losses to the resin and sample contamination with Zn leached from plastic syringes.

It was shown that the technique allows for precise and accurate determination of most of the above mentioned elements in the same 3 ml of serum sample.

Blood serum was selected for evaluation in an effort to determine the appropriateness of the intravenous hyperalimentation fluid for maintenance of the nutritional status in a population of bone marrow transplant patients. Serum was sampled weekly during the 6 weeks interval while the 15 patients were fed solely with these fluids.

Patients' serum content was compared to that of 9 clinically healthy subjects also evaluated in the study. One of the controls was sampled weekly for 3 months. These control values were stable throughout the study and well within reported normal ranges. Patients' serum analysis showed consistently higher concentrations of Cu, K, Fe and Co, and consistently lower ones of Br. Forty-two graphs illustrate such trends.

Results in the patient groups are quite different from the control values. However, no conclusive clinical conclusions can be made from the data in this study.

The method, as developed and tested for the UFTR, can be employed in precision analysis of clinical samples for nutrition studies in health and disease, environmental evaluation, animal and plant research, food monitoring for toxic levels, criminalistic and any studies involving detection of trace amounts in biological matrixes.

# CHAPTER I
# INTRODUCTION

## Object of This Study

The importance of trace elements in human health and disease has been under investigation since the 1800s. Assessment to the quantities involved, however, is only now being achieved. Modern tools, like atomic absorption spectroscopy (AAS) and neutron activation analysis (NAA) have made possible the measurement of parts per billion amounts in body tissues and fluids. Neutron activation analysis is a powerful method for some trace elements, allowing the determination of minute quantities with high precision and accuracy. Its main disadvantage is the time consuming procedure of irradiation, decay, counting, and not least of all the necessity, sometimes imperative, of having to chemically remove some interfering elements from the sample. Its high sensitivity, however, compensates for any of the above mentioned disadvantages, provided a research reactor is available.

The object of this study was to develop an accurate and reproducible method for evaluation of trace element concentrations utilizing NAA at the University of Florida Training Reactor (UFTR), which could be applied on samples meaningful in disease.

Patient samples which might be meaningfully related to the disease state and/or treatment evaluation include tissues, blood, and elimination products such as urine and feces.

Serum analysis has been largely employed for nutritional assessment and is considered to be well representative of the nutritional state of an individual with regard to trace elements. Serum can be defined as the watery portion of an animal fluid remaining after coagulation. Blood serum contains specific immune bodies such as antitoxins or agglutinins (Webster's, 1976). According to the same source plasma is defined as the fluid part of blood, lymph, or milk as distinguished from suspended material. The medium chosen for analysis was the product of centrifugation of blood and is defined as blood plasma. The word serum was used in the title for ease of future reference by other researchers, since the words serum and plasma have been employed synonymously for decades while serum is the most used. Moreover, as stated by Versieck and Cornelis (1980), serum and plasma trace concentrations are virtually the same.

Urine was chosen to sample but discontinued at the beginning of this study because of sampling difficulties and the possibility of sample contamination, as described in the methods section.

The sampling of patient's body tissues would require surgery. The scope of this study does not justify such

procedures. Therefore, blood was the only feasible and meaningful product available for evaluating the nutritional status in disease.

Protocols were designed to provide information which would allow a comparison between health and disease. Results were expected to show how much serum concentrations vary from person to person. Another control was sampled, under the same procedure, once a week for three months, to evaluate the variation with time within the same subject.

Neutron activation analysis was chosen for use in this study to evaluate trace element concentrations in blood serum from bone marrow transplant patients during their hospitalization. It was planned that patient's blood would be sampled once per week (whenever possible), and serum concentrations would be evaluated and compared with the patient's nutritional status obtained by other well established methods. A correlation is thought to exist between nutritional status and trace concentrations in blood serum, and finding it has been the goal of medical biochemistry for almost one century.

In this study, the goal was to develop a procedure to make accurate determinations of Cu, Br, K, Se, Fe, Co and Zn possible in blood serum, with the limitation that the average serum volume which could be obtained would be only 3 cc from 10 cc of blood. Eight clinically healthy volunteers each would donate 10 cc of blood for this study, and their

blood would be sampled using special Teflon(R)-made[1] needles.

When NAA is used, Na and K, and sometimes Cl contents in biological samples can mask other elements of interest. Their chemical removal is possible, but not without the risk of trace element contamination or loss of the knowledge of the true sample weight. Careful procedures, resilient testing, state-of-the-art detection and computational capabilities help to minimize these risks.

It was decided in this study to utilize a resin, called hydroxy antimony pentoxide (HAP), first tested by Girardi and Sabbioni (1968), for removal of Na from serum samples. Some researchers indicate that the resin has not worked as expected (Berry, 1979).

The procedure for trace element concentration assessment was developed with the knowledge that the NAA approach would require a fairly long turnaround time. However, the sampling technique developed can also be applied in other types of elemental analysis such as AAS when a faster turnaround is necessary.

---

[1] (R)=Registered trademark. Teflon is a registered trademark of Du Pont.

## The Disease and the Transplant

The following diseases require bone marrow transplantation as part of their treatment:

1. Aplastic anemia;
2. Acute myelogenous leukemia;
3. Acute lymphoblastic leukemia; and
4. Chronic myelogenous leukemia.

Aplastic anemia is a disease characterized by bone marrow failure with death resulting from bleeding or infection. Leukemia is a usually fatal cancerous disease characterized by excessive production of white cells, and often accompanied by severe anemia. Leukemic and/or aplastic anemia cells must be destroyed in order to cease the irreversible development of the disease and death of the patient.

Selective procedures that would strike only diseased cells have not yet been discovered; meanwhile, these diseases are treated by the total killing of the bone marrow tissue. These cells are sensitive to some chemicals and to radiation. For this reason these are the treatment modalities commonly used. However, the destruction of the bone marrow will include the killing of healthy and diseased cells alike. At this point patient receives a graft of normal cells which will initiate the repopulation of the healthy tissue.

The chemical and radiation doses must be confined to a narrow range. If the dose is too large, damage to other organs will occur; too small and some sites will not be reached and diseased cells will be left alive in numbers high enough to induce recurrence of the disease.

Reverse isolation of the patient is necessary to prevent access to infection, since most of the body's defense mechanisms are destroyed in conjunction with the leukemic/aplastic cells.

Control, isolation, and adequate nutritional balance are part of the necessary support to the patient. This work focuses on the nutritional aspect of the grafting procedure.

Patients are conditioned with chemotherapy (CY), 200 ng/kg cyclophosphomide to eradicate their immune response to the marrow graft previous to a 600 rem midline tissue dose of total body irradiation (TBI). Donor marrow is infused intravenously 1 to 18 hrs after TBI, or 36 hrs after the last CY administration (Glucksberg et al., 1974). TBI and CY will destroy the leukemic and/or anemic cells, as well as major portions of the normal ones.

The donor cells are injected intravenously, and travel through the patient's circulatory system until they adhere to the bone marrow, where they "stick" and begin to proliferate, producing enough cells to give a normal blood count within a month.

Marrow donors are normally siblings who were matched with the patients at the major histocompatibility region (MHR), as determined by serological identity for HL-A antigens and by nonreactivity in leukocyte culture (Glucksberg et al., 1974).

Bone marrow is aspirated from the donor under a general anesthetic from the anterior and posterior iliac crests and the sternum. The donation is in the region of 300 to 1000 ml depending on the weight of the recipient and the cellularity of the donor's marrow (Barrett, 1980). The donor normally leaves the hospital the day after the donation.

The graft-versus-host (GVH) disease is a complex clinical syndrome which reflects the infectious complications related to the compromised host immunity, due to TBI and CY (Glucksberg et al., 1974). The engrafted marrow sometimes contains immunologically competent cells that will produce a GVH reaction, which is a major impediment to successful transplantation of allogenic marrow. Only 60% of the bone marrow transplants succeed, and GVH disease, as well as the sometimes inadequate nutritional support in the months before immunity is fully established (Barrett, 1980), is considered a major cause for this percentage not being higher.

Graft-versus-host disease may appear in many organs, and in different degrees. There may be skin changes,

degeneration of mucosal glands, degeneration and necrosis of small bile ducts, or even mild changes on skin, gut, liver, and the lymphoid tissues.

Graft-versus-host disease, when it occurs, takes from 7 to 30 days to onset (Glucksberg et al., 1974).

Figure 1.1 shows the usually employed bone marrow transplantation procedure.

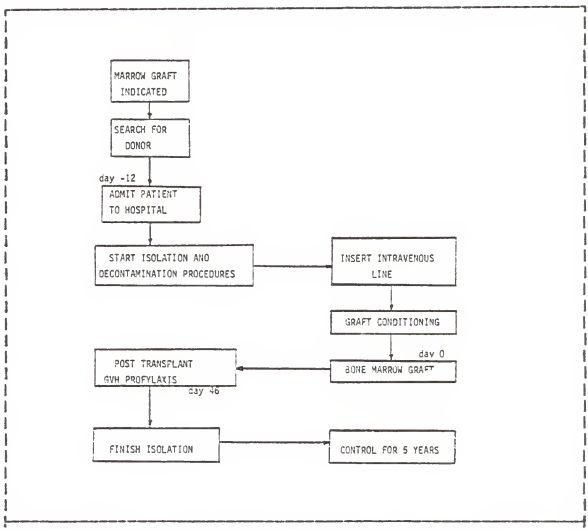



Figure 1.1. Usually employed bone marrow transplantation procedure.

## Nutritional Aspects of the Bone Marrow Transplant

Extensive oral mucositis and gastroenteritis will result from the high dose CY and the TBI. As a consequence, a markedly decreased oral caloric intake results, and most of the patients temporarily stop all oral caloric intake (Glucksberg et al., 1974). It is not known to what degree high doses of CY and TBI affect the nutritional balance. Almost all patients suffer from severe oral mucositis and show dramatic weight loss and marked signs of malnutrition. Infections with or without fever, and GVH disease, may also have negative effects on the patient's ability to maintain an optimal nutritional condition.

Total parenteral nutrition (TPN) is administered intravenously and can successfully overcome most of these problems which frequently complicate the course of bone marrow transplantation (Schmidt et al., 1980).

> TPN is an established technique of nutritional support which can potentially provide vitamins, minerals, aminoacids, carbohydrates, and occasionally fat in amounts necessary to prevent or reverse the state of malnutrition. (Layton et al., 1981, p.127)

## Trace Elements in Nutrition

Trace elements are known to have important effects on the physiological processes which occur in all living systems. Several investigators have shown that a definite relationship exists between trace element levels in the body and certain diseases. There is also strong evidence that

some types of cancer are associated with abnormal trace element levels. According to Mertz (1981),

> An element is essential when a deficient intake consistently results in an impairment of a function from optimal to suboptimal and when supplementation with physiological levels of this element, but not of others, prevents or cures this impairment. (p. 1332)

The determination of whether an element is essential is based on the following criteria (Cotzias, 1967):

1. The element is present in all healthy tissues of living organisms;
2. The concentration from one animal to the next is fairly constant;
3. Withdrawal from the body induces,reproducibly, the same structural and physiologic abnormalities regardless of the species studied;
4. Addition either presents or reverses these abnormalities;
5. The abnormalities induced by deficiency are always accompanied by pertinent, specific biochemical changes; and
6. These biochemical changes can be prevented or cured when the deficiency is prevented or cured. (p. 5)

Recent advances in analytical techniques now make it possible to measure trace elements more rapidly and with greater accuracy in biological fluids, thus allowing the use of this parameter for management of certain diseased conditions in man. Nutritionally speaking, it has become obvious that the availability of trace elements in the human diet is a complex problem which needs a considerable amount of research.

Some essential trace elements are still not included in TPN fluids, simply because too little is known of their requirements to allow rational formulations to be made. One philosophy holds that it is better to err on the side of deficiency than to risk toxicity. Also, very long-term hyperalimentation is likely to produce other deficiencies (Faulkner, 1981).

The following are statements made by the Nutritional Advisory Group of the American Medical Association (Faulkner, 1981):

1. Certain trace elements are needed in parenteral fluids for maintenance of good nutritional status and for the nutritional rehabilitation of patients;

2. The trace elements now recognized as essential for humans are Co (as vitamin B12), Cu, I, Fe and Zn;

3. There is evidence--although fragmentary--of the need for Cr and Mn;

4. Fluorine reduces tooth decay, but has no other known function;

5. Information about the nutritional aspects of As, Mo, Ni, Se, Si, Sn, and V is insufficient to warrant a recommendation for their inclusion in parenteral fluids; and

6. Parenteral solutions contain Cu (0.4g/l), Cr (0.004 g/l), Mn (0.1 g/l) and Zn (1.0 g/l).

The dependence of the severity of signs and of the effects of resupplementation on the degree of deficiency was formulated mathematically by Bertrand in 1912 (Mertz, 1981).

Trace element research is currently based on two conclusions, both drawn from the Bertrand's Model (Mertz, 1981):

1. For each element there is a range of safe and adequate exposures, within which homeostasis is able to maintain optimal tissue concentrations and functions; and

2. Every trace element is potentially toxic when the range of safe and adequate exposure is exceeded. (p. 1333)

A better understanding of many diseases will result from more knowledge of trace element content in various organs and components of biological systems in different disease states. A synthesized description of the known physiological roles of each trace element under discussion in this study is presented below:

Bromine

Little information is available on Br metabolism. The element has not been conclusively shown to be involved in any essential function (Versieck and Cornelis, 1980).

Cobalt

The only known biochemical function of Co is as an integral part of vitamin B12, which forms a cofactor for the activation of methylmalonyl-CoA. It is physiologically involved in hematopoiesis. Its absorption is increased in Fe deficiency and is depressed by high Fe concentrations in diet. Cobalt must be absorbed as vitamin B12 by humans, since this cannot be synthesized from Co.

Copper

No instance of Cu deficiency in adults has been reported that has not resulted from repeated and prolonged diarrhea plus a diet very poor in Cu.

Parenteral administration of Fe fails to alleviate anemia, but it increases Fe stores in the liver and reticulo-endothelial system. These observations can be explained by the assumption that Cu is essential for the release of Fe from the intestinal mucosa and the Fe storage tissues.

In man, hypocupremia is associated with nephrosis and Wilson's disease, both of which are accompanied by increased urinary excretion of Cu (Underwood, 1971). Hypocupremia occurs in many disease conditions in man. The cause in these cases is not a dietary deficiency of Cu. It can be related to a defect in ceruloplasmin synthesis, poor absorption, excessive excretion, or some other disturbance associated with the disease (Underwood, 1971).

Copper is a component of cytochrome oxidase and other metalloenzymes. It is involved in the absorption and utilization of Fe in the synthesis of hemoglobin and myoglobin and plays a role in the formation of melanin and myelin and in the maintenance of neurologic functions.

It is transported mainly in loose binding with albuminum (Faulkner, 1981).

Iron

Iron is a component of hemoglobin, myoglobin, catalase, and other enzymes and is involved in transport and utilization of oxygen. For transport, Fe is bound to serum protein (transferrin) and its storage occurs in heme proteins. Iron deficiency is frequent in infants, pregnant women and teenage girls and manifests itself as anemia (Faulkner, 1981).

Selenium

Selenium has been shown to be an integral part of the human red cell enzyme glutathione peroxidase, thus establishing its essential role in man (Prasad, 1976).

Neutron activation analysis and molecular fluorescence are now the most common methods of Se analysis (Olson, 1976).

The physiologic function of Se is supposed to involve protection against accumulation of hydrogen peroxide, i.e., an antioxidant function. Experimental and epidemiologic evidence indicate an inhibitory effect on carcinogenesis. Concerning its metabolism, the absorption is in the form of selenite (40-70%). Selenium deficiency has been produced in animals, and hemolytic anemias have been reported in humans (Faulkner, 1981).

Zinc

Zinc has been recognized to be essential for animals, and its deficiency in man has been also observed. The nutritional availability of this element poses serious problems in certain segments of the human population. The important role of Zn in growth and gonadal function and in nucleic acid metabolism has been recognized only during the last 15 years. The requirements of Zn may vary widely depending upon the phytate and fiber content of the diet.

The importance of interaction between various elements is exemplified by the adverse effect of Cd (a toxic element) on the metabolism of Zn (an essential element) in man (Prasad, 1976).

Zinc is a component of at least 30 metalloenzymes. It is involved in the synthesis of RNA and DNA, connective tissue and glutathione. Its transport is primarily done through the plasma albuminum, with 60-70% bound to it. Zinc deficiency is correlated to growth retardation, impaired sense of taste, hypogonadism and slow wound healing (Faulkner, 1981).

## Trace Element Studies for Serum

Several studies have attempted to improve the precision and accuracy in trace element determination in human blood serum. Experiments usually involve apparently healthy individuals and have the ultimate goal of determining

nutrition reference values for comparison with other special conditions like disease, pregnancy and even geographical location of the populations studied.

A very complete review on the subject was published in 1980 by Versieck and Cornelis, who observed

> Serious inconsistencies in the reported concentrations of trace elements in plasma or serum of healthy individuals . . . . (p. 247)

They also stated that

> There is solid experimental evidence that much of the existing controversy is due to inadequate sampling and sample handling or to defective analysis . . . . There is increasing awareness that specimen contamination is responsible for a considerable number of erroneous values . . . No program of low level trace element analysis should be started before adequate contamination control procedures . . . have been developed (clean room facilities, container cleaning technique, sample collecting and handling devices). There is growing consensus that some of the controversy may be due to systematic errors. Thus, a major trend should be to practice quality control by the regular use of reference materials . . . . (pp. 217, 247)

Several homeostatic control mechanisms exist in living organisms. Essential elements tend to undergo such control in order to maintain the required levels in the organism (Bertinchamps et al., 1966).

Healthy individuals who are not environmentally exposed to trace elements should present concentration values with a rather small range (Versieck and Cornelis, 1980).

The values presented in the following paragraphs were extracted from a list of experimental results (Versieck and Cornelis, 1980), and suggest values which would represent the present status of blood serum research.

Bromine

Behne and Jurgensen (1978) found 4.5 µg/ml of Br in serum from supposedly healthy subjects.

Cobalt

The published plasma or serum values are in good agreement. Versieck et al. (1978), for example, found the Co serum concentration to be 0.108 ng/ml.

Copper

Copper is involved in activities as diverse as hemoglobin synthesis, connective tissue development, and normal function of the central nervous system (Versieck and Cornelis, 1980). Olatunbosun et al. (1976) determined Cu concentrations in serum using atomic absorption spectroscopy and found 1.01 µg/ml.

Iron

Versieck et al. (1977) found 1.63 µg/ml of Fe in serum, averaged from 36 apparently normal individuals. Blood was sampled after an overnight fast.

Selenium

Selenium is an essential constituent of glutathione peroxidase, and is likely to be implicated in other enzymes as well. Sullivan et al. (1979) reported 0.12 µg/ml for Se in blood serum of clinically healthy subjects.

Zinc

Zinc is a constituent part of more than 30 metalloenzymes, and is related to both nucleic acid and protein metabolism. Davies et al. (1968) published 0.96 µg/ml as the serum Zn concentration in humans.

The University of Florida performed serum analysis for trace elements (Maenhaut et al., 1981) using PIXE and NAA. The human serum samples for that study were taken from patients with hemochromatosis. Some of their results are listed below:

| | |
|---|---|
| K | 187 +- 11 µg/ml |
| Fe | 2.6 +- 0.07 |
| Co | 0.0018 +- 0.0001 |
| Zn | 1.1 +- 0.04 |
| Se | 0.123 +- 0.020 |
| Br | 4.57 +- 0.18 |

Attempts have also been made in the Netherlands, by Woittiez and Das (1982), to analyze serum with removal of Na and Cl prior to irradiation. Strong contamination of the samples was observed in this case, produced by the presence of Sb leached from the ion exchange resin. According to analysis performed by Nakahara et al. (1979), cancer patients showed below normal concentrations in Rb, Fe, Co, Cu, Zn, Al and Se. Downs syndrome patients were found to have similar deficiencies in Cr, Mn, Fe, Co, Zn, Cu and Sb.

Kostadinov and Djingova (1981) published results from serum analysis using thermal and epithermal NAA and presented a discussion of the technique employed. A list of 265 references on works involving serum or plasma concentrations of trace elements accompanies a paper by Versieck and Cornelis (1980).

Quantitative Analysis through Neutron Activation

An important application of nuclear science is the use of induced radioactivity as a mean of chemical analysis. It has come to be known as activation analysis and makes use of primary particles such as neutrons, photons and charged particles to activate the atoms to radioactive species, whose disintegration characteristics are subsequently determined and used as a basis for identifying the elements originally present in the sample.

The nuclear species can be identified according to half-life, type of radiation and energy spectrum.

Slow neutrons are the most commonly employed activation particles, producing reactions of the radioactive capture or (n,gamma) type. Slow neutron sources most commonly employed are nuclear research reactors.

An example of activation nuclear reaction is shown below:

$^{23}Na(n,gamma)^{24}Na$---(15 hrs)---- $^{24}Mg$ + beta + gamma

The product nucleus $^{24}Na$ is radioactive, emits gamma rays and has a half-life of 15.02 hrs. This technique is called thermal (or slow) neutron activation analysis, or simply neutron activation analysis, or NAA.

In certain situations it is necessary to perform chemical separation in conjunction with activation in order to achieve a higher degree of sensitivity.

In other situations the use of a protocol involving irradiation time, decay time and counting time alone can give a sufficiently adequate analysis quality, and chemical separations become unnecessary. This technique is known as instrumental neutron activation analysis, or INAA.

The University of Florida Training Reactor--UFTR--is an Argonaut type research reactor. It has a considerably stable thermal flux which makes it adequate for long studies in NAA. The maximum power is only 100 kW, which yields a thermal neutron flux in the order of $1 * 10^{12}$ neutrons/$cm^2$s. Most modern research reactors operate with a thermal flux of $10^{13}$ or higher neutrons/$cm^2$s which allows for the use of smaller samples and/or shorter irradiation times.

The UFTR has two inherent limitations when it is used for activation analysis:

1. The samples have to stay in the reactor for longer periods of time because of its low neutron flux; and
2. The operation is not continuous. If several days of irradiation are necessary, such will have to be accomplished in daily steps.

For short irradiations the reactor is equipped with a pneumatic transfer system. Counting facilities are in an adjacent radiochemistry laboratory.

## CHAPTER II
## METHODS--SPECIMEN COLLECTION

### Sampling

Many researchers have recently emphasized the necessity for an adequate sampling protocol which would involve no trace element contamination of the sample, and which would maintain it contamination-free until analysis is completed. Studies at the University of Ghent, Sweden, by Versieck et al. (1973, 1977, 1978, 1980, 1981), have been particularly directed to contamination assessment during sampling procedures, sample storage and furnishing suggestions for proper sampling.

Attention has also been given to this subject in the U.S.A., as can be seen in papers by Speecke et al. (1976) and Moody et al. (1977). There is evidence to affirm that many past trace element studies have not received the necessary attention to sampling. Their conclusions are, therefore, subject to reexamination, since the improper sampling might have led to unreliable results (Versieck et al., 1978).

Urine was chosen to sample but discontinued at the beginning of this study because of sampling difficulties and the possibility of sample contamination, as described below:

1. The urine would have to be collected in acid-cleaned polypropylene containers free of contact with any other materials. Hospital protocol requires urine volume records to be taken for these patients, which requires the collection of the sample in a special container and its pouring into containers provided with a volumetric scale.

   This procedure can potentially cause sample contamination with trace elements, since the equipment used here is not specially cleaned for procedures involving trace element studies. The same limitation was associated with feces sampling.

2. Freeze-drying tests have been performed with urine, and the procedure is very time consuming, not adequate for a large scale-study, or quick assessment of information. Future studies should attempt low temperature ashing for the sample reduction step.

Blood sampling is a special case since it involves the use of syringes and needles. Researchers report a wide range (1000 fold) of concentrations for several elements in serum of clinically healthy subjects (Versieck et al., 1978).

Further studies showed that proper sampling procedures could bring those concentrations down to lower values, suggesting that the higher concentrations previously obtained were due primarily to sample contamination.

Hospital personnel, who usually are authorized to perform the blood withdrawals, must be aware of a series of details involving sampling in trace element studies. The blood sample must be kept as free as possible from anticoagulants, silicon lining, and other chemicals usually employed in blood chemistry, and with which hospital personnel are familiar. This familiarity could lead to improper sampling, through the use of non-treated syringes or containers, vacutainers(R) or needles.

The presence and advising of the analyst during sampling was followed in this study and is strongly recommended. A written procedure should also be permanently posted at the nursing room for consultation.

## Sampling Technique

### Syringes

Bone marrow transplant patients at Shands Hospital usually stay at the Bone Marrow Transplant Unit (BMTU) for less than 2 months. Most had their blood sampled once a week for the purposes of this study. Control samples from healthy subjects were also collected and analyzed, bringing the total number of samples to 80.

Two hundred syringes (Monoject(R)--12 cc), all manufactured on the same day, i.e., same serial number, were acid washed for optimal sampling conditions. The washing procedure consisted of one week in 1:3 nitric acid

bath, and then washing and rinsing with ethyl alcohol and deionized-distilled water. The syringes were cleaned separately from the internal stopper, since its rubber tip tends to deteriorate in acidic environments and leave residuals inside the blood sample. The stoppers were cleaned with paper tissue, for the removal of the silicon grease that comes with it and then washed with distilled-deionized water.

The syringes were not dried but immediately stored inside air-tight heat-sealed plastic bags until the time of sampling. They were rinsed with deionized-distilled water immediately before sampling. After being washed, the syringes were plugged with clean polyethylene caps, which were changed after sampling.

The plastic bags served two purposes: first, they kept syringes from receiving trace element contamination from the surrounding environment and, secondly, they kept them sterile, a necessary condition for any apparatus which is allowed to enter the BMTU.

Needles

The use of needles is a subject of controversy. Hospital personnel avoid the use of needles because it causes added trauma to patients. This is particularly important for patients in the BMTU because they already receive many daily injections. To facilitate these procedures, an intravenous

(I.V.) feeding line is used. This line is implanted in the patients and kept in place until they are discharged from the hospital. The line leads straight to the heart and would be perfectly suited for blood sampling except for three aspects:

1. The line stays busy 18 hr a day during I.V. feeding. Any sample taken during this period would contain trace elements from the I.V. fluid that would have been attached to the walls of the line, and it would be non-representative of the patient's real blood picture with regard to trace elements. In addition, during that period, the blood becomes contaminated with I.V. fluids which contain trace element amounts. Therefore the samples do not represent the true nutritional state of the patient.
2. Also, for reasons stated above, the blood should not be sampled right after the 18 hr I.V. feeding.
3. The I.V. line is closed for 6 hrs every day ("window") following the 18 hrs I.V. period. An anticoagulant (3 ml) fluid is injected into the line, and stays there until the next I.V. period (time III) as shown in Figure 2.1. Time III was initially chosen for blood sampling through the same broviac line.

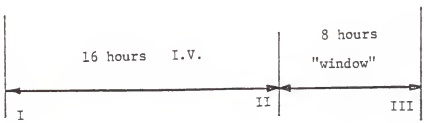



Figure 2.1. Daily normal I.V. schedule for BMT patients.

Time III advantage: After 6 hrs of no I.V. injection, the trace element concentrations in the blood should have equilibrated with all body fluids and therefore will be better representative of the overall nutritional condition of the patient.

Time III disadvantage: Possible contamination might still occur from the anticoagulant fluid, in spite of the fact that it is previously flushed from the line by means of a 5 ml blood waste sample.

In December, 1982 the procedure for making the I.V. fluid was changed in order to allow a better assessment of the real situation of the trace elements in their serum specimens. The I.V. fluid was originally prepared using

stainless steel needles, which were suspected of causing sample contamination which would overestimate the concentrations in the I.V. solutions and possibly interfere with any intended correlation between blood levels and I.V. levels. Angiocath(R) needles, which are non-metallic, were put into use in the preparation of intravenous feeding solutions.

Controls blood was sampled using special Teflon-made needles. According to Versieck et al. (1980), steel needles are responsible for large additions of trace metals to blood samples. Their work included neutron-irradiated needles which were then used for blood collection. The collected material showed that the serum, when counted on a germanium detector, contained many radioactive metals. Teflon(R) needles contain insignificant amounts of such metals, and are believed to eliminate such source of contamination during sampling (Versieck et al., 1980).

Sample Preparation

The blood samples were quickly taken from the hospital to the preparation room, which is equipped with a laminar flow hood[1] strongly recommended for this kind of operation.

---

[1] These hoods are specific for sample handling. Filtered air is constantly blown on the sample handling area in order to keep samples, containers and instruments from deposition of airborne dust particles.

The blood was centrifuged inside the syringe, in order to avoid transfer and associated contamination risks. Several centrifugations are necessary in order to separate as much serum as possible from the packed blood cells. Only 1 ml of the 10 ml initially collected is discarded in this procedure. The samples are collected in 10 ml snap cap hinge containers made of polypropylene, which is the material, along with polyethylene, best suited for trace element studies, according to studies by Moody et al. (1977). These materials have been shown to leach the smallest trace element amounts when compared with other materials commonly employed in the manufacture of vials and containers.

Freeze-drying is then performed on the samples in these same containers in order to keep the sample chemically isolated from the laboratory environment.

Glass containers were never employed since they contain a number of metal contaminants such as Mn, Au, Cu, Ga, La and Sb (Gills et al., 1970) which have been shown to leach into the sample.

Non-powdered surgical gloves were worn throughout procedures of syringe washing, handling and sample preparation, in order to keep them free from any of the trace element contaminants present in the perspiration from the hands.

Surgical masks as well as safety glasses were also worn in order to protect the face and eyes against acid spills

and viruses which the samples might contain. They also serve to protect samples from deposition of contaminants resuspended in air due to breathing.

After sample collection, the syringes are sometimes placed inside plastic bags (double-packed) by hospital personnel, indicating that the possibility of virus contamination exists. According to oncology physicians, leukemia contamination is not likely to occur.

## Protocol Description

The samples were carefully collected from bone marrow transplant patients at Shands Hospital, Gainesville, Florida. Special authorization for experiments involving human subjects must be granted by the medical staff of that hospital, under the strict regulations that govern procedures that those patients should undergo. Only 10 ml per week were permitted to be withdrawn from each adult patient. Only 6 ml were drawn from children under ten years. Those limitations are well justified by their already delicate (fragile) nutritional condition. All research protocols were designed to interfere as little as possible with the patients status since they are already under stress from many other procedures. Ten milliliters would give, after centrifugation, 3 ml of blood and 3 ml of serum. After freeze-drying the dry weight would on the average amount to 350 mg, an amount by itself in the lower

quantitative limit for the neutron activation procedures chosen to evaluate the sought elements.

As part of the approach adopted in order to manage the difficult blood serum analysis, special cleaning procedures and contamination control were of the highest priority for all sample and labware handling. A brief description of these procedures follows for each sample:

1. Plastic disposable syringes were all acquired from the same batch, so that they would have the same manufacturing characteristics, no biases being then expected in any comparative trends between samples.
2. The syringes were cleaned in acid bath 1:3 nitric acid for 1 month, then individually rinsed in double distilled-demineralized water. Polyethylene vials underwent the same treatment and were allowed to dry in a hood equipped with laminar-flow-HEPA-filtered air.
3. The syringes were then individually packed in polyethylene bags and heat sealed, to avoid air-particulate contamination of inner surfaces. A PVC-syringe-cap kept each syringe air-tight.
4. Once a week, blood was withdrawn from 2 patients (average number of BMT patients who had their blood sampled each week).
5. The samples were taken immediately to the laboratory, centrifuged inside the syringe in order to avoid

trace-element contamination, and then dripped, serum and blood, into two different polyethylene vials. The vials were all numbered, and marked with the date and patient's name. Each serum or blood drop was individually checked for blood or serum presence, respectively. The centrifugation procedure takes about 1 hr for each sample.

6. The snap cap vial was then weighed, sealed with parafilm and frozen for further freeze-drying.
7. Seven-days-freeze-drying (a batch procedure, for each 20 sample group) was performed at -70°C and 60 microns of Hg pressure, and samples were then reweighed, resealed with parafilm and stored in a clean and dry place.

For freeze-drying, the samples were opened, a small filter paper placed and secured with a rubber band on their mouth, and frozen with liquid nitrogen. These procedures avoided boiling during the low pressure freeze-drying while maintaining the samples isolated from each other after their turning into powder.

Water loss is then calculated, and the sample wet-dry weight ratio determined. All information was recorded in a logbook.

Samples were then irradiated for 28 hrs with the $1*10^{12}$ neutron/cm$^2$s thermal flux of the UFTR at 100 kW power.

8. Twenty-four hours after irradiation the samples were taken from the reactor cell and brought to a shielded fume hood, where they were digested in 12N HCl and then passed through 2 g of HAP. The use of HAP is to remove the highly activated Na present in the sample.

   This procedure has to be accomplished in a short period of time, since some of the sought-after elements have activation products with short half-lives like $^{66}Cu$, $^{42}K$ and $^{82}Br$.

9. Samples were then counted twice each, first for the determination of highly activated short-lived radionuclides. This first count should take place 48 hr after the end of the irradiations.

   The second count searched for longer-lived elements, clearly seen after the decay of shorter-lived radionuclides. Such a condition would occur 25 days after the end of the irradiation.

10. Analysis would then proceed by measuring known amounts of Cu, Br, K, Co, Se, Fe, Zn and Cr as primary standards in a comparative methodology, called the comparator method, which is described in Appendix A.

With such small samples available, good precision and accuracy would only be attainable by using long enough irradiation schedules. Adequate precision was obtained in 16 hr irradiation, but since so much time and material was

involved, and since the necessary precision for trace element concentration determinations was not known, a longer irradiation time was a clear alternative for more precise and accurate results. Twenty-eight hours irradiation was chosen as the irradiation time to be used for the serum samples in this study.

Only very small blood samples were available, for reasons stated above, while several (7) were the sought-after elements. Such limitations (sample size and number of sought-after elements) demanded extreme cleanness during sampling and analysis and the need for handling of highly radioactive materials. The need also arose for chemical treatment and separation of the sample and for 2 countings of each sample after irradiation. Eighty-four samples and 24 standards were irradiated in 8 batches of 12 samples each. Each batch was irradiated for 3 consecutive days. Each sample or standard was counted for 2 hrs and then again for 5 hrs, 3 weeks later, producing 216 spectra and 756 data points.

# CHAPTER III
# METHODS--SERUM ANALYSIS

## Irradiations

### Selection of Optimal Procedure

Several tests were performed in order to determine what would be the optimal procedure to be followed for serum samples--particularly with regard to their high Na content--when analyzing for such trace elements. Five possible irradiation and chemical separation schedules were attempted as follows:

1. Short time irradiations. Dry serum test samples were irradiated for 15 seconds in the pneumatic transfer system of the UFTR and then counted on an intrinsic germanium detector. The sought-after peaks could not be observed because of the presence of the interfering Na spectrum.
2. Sodium extraction after a short time irradiation. It was expected that a short irradiation would produce several short-lived nuclides, and that the elimination of the Na interference would allow them to be quantitated. The HAP resin, discussed earlier in Chapter I was designed to hold Na atoms at a very low pH and allow all other elements to pass through.

The resin, however, proved to work slowly. A few hours were required per sample after which time no short-lived nuclide activity remained.

3. Sodium extraction before a short time irradiation was then attempted following a procedure reported by Nyarku and Chatt (1982) for sea water analysis. Sea water is well suited for this procedure primarily because large sample volumes can be obtained and little treatment is necessary before passing it through the resin. Small serum samples, however, suffer from the fact that they can be easily contaminated by glassware, acids, and even the resin, which contains large amounts of Sb.

4. Chemical separation prior to long irradiations. Sodium was removed from the samples using the HAP resin. After that, they are in liquid form, dissolved in 12 N HCl, and probably contaminated with some resin material, as well as from the glassware. Plastic funnels and beakers, made of polyethylene or polypropylene were used, to avoid metal contaminants likely to occur with the use of glass. Wet samples cannot be sent into the reactor if not properly contained. Quartz vials are extensively used as containers, but weight control of the sample turns out to be difficult to maintain. A test was performed with a sample which had been irradiated for

16 consecutive hours. No Na was present in the sample; therefore it could be counted a few hours after irradiation without concern for excessive radiation exposure. However, several peaks in the sample were related to $^{122}Sb$ apparently leached from the resin. Such interferences were very strong, and this procedure did not appear to be adequate. Work was done (Nyarku and Chatt, 1982) in order to reduce leaching of Sb in these situations with limited success.

5. Chemical separation after irradiation. This technique has the disadvantage of high radiation doses associated with the sample handling after irradiation. It requires detailed planning to avoid unnecessary exposures and organization of work in case several samples are to be prepared simultaneously. For large sample sizes, precipitation methods can be used in order to obtain significant amounts of the sought-after elements. Such methods cannot be used, however, if only a very small sample is available and if many elements are under investigation.

Instead of working in a way to select what was to be measured, as it normally occurs in trace analysis, this study concentrated first on eliminating the interfering Na and then focused on the trace elements

remaining in the sample. For this reason, the use of the HAP resin became an essential part of the procedure.

Several elements could be detected with acceptable precision, but to achieve even more accurate results the irradiation time was increased from 16 to 28 hours.

The potential for precise determination of several sought-after trace elements in these serum samples made the fifth procedure the one chosen for analysis. Radiation exposures were believed to be of no great concern in a well applied program with pre-planned and carefully executed procedures.

Irradiation Scheme

Irradiations were performed in 3 daily steps, totalling 28 hrs for each set of samples.

Sixteen hours after the end of the irradiation, the samples, in batches of 12, were recovered by the experimenter and digested in acid (12N HCl). After passing them through the resin, which required 12 hrs, they were Na-free and ready for counting. In order to obtain results on Cu, Zn, Br, and K, a 3-hour count was necessary. Twenty-five days later, the samples were counted again for 5 hrs each to obtain accurate statistics for Zn, Se, Fe and Co.

The same procedure was followed for the primary standards prepared in the laboratory. Performing NAA by the comparator method requires that standards and samples be irradiated and counted under the same conditions. Appendix B describes the method and how it was applied in this research.

Resin Tests

Two tests were carried out in order to evaluate the performance of the resin. Known amounts of these elements were irradiated and then diluted in 12N HCl. The solution was counted on a Ge detector, then passed through the resin, and counted again. If the resin cannot adsorb the sought-after elements in significant amounts then the counts under their activation peaks after the resin treatment should equal the ones before the treatment.

As seen below, HAP proved to work well when one of the standards was irradiated, digested in acid and counted for Cu, K, and Br, then passed through the resin and counted again. The closeness of the numbers shows that the resin does not adsorb significant amounts of Cu, K or Br, as shown on Table 3.1.

Table 3.1. Counts under activation peaks before and after HAP elution.

| Element | Before HAP elution (counts) | After elution (counts) |
|---|---|---|
| Br | 359 +- 10.2 | 351 +- 10.7 |
| Cu | 13.6 +- .0396 | 13.7 +- .0289 |
| K | 0.194 +- 0.00831 | 0.165 +- 0.0063 |

The resin seems to work efficiently for these three elements. To evaluate the behavior of the resin with regard to Zn, Fe, Co, Cr and Se, the contents of one set of 12 spent columns was counted on the detector after a decay period of 3 weeks. The object was to check for the presence of such elements, which would indicate that a non-quantitative elution had taken place.

The column used in such tests did not have the filter paper positioned above the resin. The filter paper was only used after the second batch of samples as described in Appendix B. This evaluation, therefore, produced conservative results. It was concluded that, on the average, for each serum sample that passes through the resin, less than 10% of the Se was retained. All Zn passes through, as well as more than 99% of Fe, 99% of Cr and 99.9% of Co.

Contamination Assessment

The sample has contact with only two types of material: syringes and storage vials. A test was performed in the early stages of the study with the objective of evaluating possible sample contamination sources. A qualitative determination of the syringe and black stopper material revealed a high content of Zn.

Further studies showed evidence of sample contamination with Zn. Four acid cleaned containers were used, 3 of them containing pure water (distilled and deionized) alone, pure water which had been in contact with the interior of the syringe for 30 minutes[1] and pure water which was shaken inside the syringe for the same 30 minutes.

The three water samples were freeze-dried and irradiated for 5 hrs, along with an empty vial which served as a blank.

The results made evident the lack of contamination by any of the sought elements. Zinc peaks, however, showed the following numbers of counts:

| | | |
|---|---|---|
| 1. | Empty container (blank) | none |
| 2. | Pure water container | none |
| 3. | Pure water 30 min in syringe | 160 |
| 4. | Pure water shaken 30 min in syringe | 740 |

---

[1] The blood samples would stay in the syringes for an average time of 30 minutes, comprising sampling, transportation, centrifuging and serum separation.

It was concluded that the syringe environment introduces Zn into the samples. The introduced amounts are significant when compared with the levels expected for human serum. Such contamination is in the order of 0.3 ug/ml, or 15% of the normal Zn levels.

Primary Standards

Two sets of primary standards were made, one for Cu, K and Br and another for Se, Fe, Zn and Co. The first set contained quantitative information for the short lived elements Cu, Br and K. This set was analyzed two days after irradiation. The second set was equally diluted to a standard volume, however it was counted only 25 days later. They were kept separated to avoid interferences, high matrix background and because of practical limitations in their production.

The elements used were obtained from the Department of Chemistry. Some elements were obtained in elemental form, while others were only available in the form of compounds. The compounds used for the production of standards are listed below:

1. Potassium chromate
2. Ferrous ammonium sulfate hexahydroxy
3. Cobalt sulphate
4. Zinc
5. Potassium hydroxide

6. Potassium bromide

7. Red selenium (amorfous)

Each set consisted of 12 plastic bags, each containing 100 ul of standard solution, which contained known amounts of each sought trace element.

The bags were heat-sealed and placed inside a second bag, also made of polyethylene. The second bag was used in order to avoid contamination to the external surface of the internal bag, which later was immersed in water to bring the trace element amount up to the 75 ml beaker geometry for counting. In this way, when the external bag was discarded, the internal one was virtually free from contamination.

One bag containing the standard solution for Cu, Br and K was placed inside the irradiation vial along with the second bag containing the Se, Fe, Zn and Co. A total of 24 bags were placed inside the 12 vials to utilize the 12 irradiation positions assigned according to the analysis design discussed in Appendix A.

These standards underwent the same irradiation, decay and counting procedures assigned to the samples which occupied the same respective positions inside the reactor. Positioning was again important, and the standards were numbered from 1 to 12 for Cu, Br and K and 13 to 24 for Se, Fe, Zn and Co.

The bags were carefully opened with clean scissors and the scissors were then washed above the geometry beaker with

distilled-deionized water so that this water and any trace elements within could be recovered for quantitative determination. The bags were throughly washed with distilled-deionized water, and after that placed on the detector to check for any Cu, Br or K that could have been left in the resin.

Copper and K were never found in this procedure, meaning that all the amounts initially transferred from those standard 100 ul were now diluted to the standard counting geometry. Bromine, however, could be detected, suggesting that some of it may have become attached to the bag walls. This behavior has been previously observed and commented on by some researchers (Woittiez and Das, 1982).

Bromine and Sb peaks were clearly identified in the external bag radiation measurements indicating that these elements are present in the bag material. The experimenter had his gloves also counted, and Sb was found on them. Polyethylene bags, as concluded, will leach Sb after long time irradiations.

One of each set of standards was diluted in 12N HCl instead of water. As described in the previous section, the intention was to test for resin properties, more specifically, non-adsorption properties of the resin concerning the sought-after trace elements, since it is desired that all trace elements be able to pass through the resin without being adsorbed. Standards were prepared in

such a way that the amount of trace elements placed in 100 ul of stock solution would be approximately the ones expected, according to values compiled by Versieck and Cornelis (1980), in 3 ml of serum.

HAP

As stated previously, biological samples contain high levels of Na, which is readily activated during irradiation and can become a major interference in neutron activation analysis. Sodium-23 has a high thermal neutron cross section and comprises 100% of stable Na thereby producing a large amount of $^{24}$Na with a half-life of 15.02 hrs. This produces significant interference in samples irradiated for long periods of time (i.e. hours).

Such interference can be observed even in short-time irradiations, for example a few seconds, although not as markedly as in long-time irradiations. In the latter case other long-lived elements can be detected only after the decay of the activated Na. One hundred milligrams of dry serum, activated for 2 days, requires a decay time of 2 weeks before Fe, Se, Zn, and Co can be detected. Shorter-lived elements like Cr cannot be measured in this situation.

Hydroxy Antimony Pentoxide (HAP) was originally developed by Girardi and Sabbioni (1968) in order to remove Na from samples. This is achieved by passing samples diluted in very acidic solutions through the resin. Very low pH samples

are a must if Na is to stay in the resin while high percentages of other elements pass through, according to experiments by Krishnan and Crapper (1975).

Unfortunately, the procedures involving the design of experiments, resin preparation and use are not detailed in available references.

As was determined in this study, some important details must be observed if the resin is to work as expected. These are described in Appendix B.

The properties which are important for the successful operation of the resin are

1. The resin should hold Na and allow other elements to pass.
2. It should be resistant to high concentrations of acids and high radiation exposures.
3. It should work fast to keep radiation doses to the experimenter as low as possible.
4. The resin should have a high retention capacity (i.e., 30mg of Na per gram of resin). The higher the retention capacity, the smaller will be the amount of resin needed. This is a desirable condition since the resin cost is relatively high, ($230 per 500 g). Each assembled column contains approximately 2 g of resin.
5. It should have a high percentage of large granules. Small grains will offer a larger adsorption surface per unit of weight, but they often cause clogging of the resin.

The resin, actually an inorganic ion exchanger, worked adequately for the purposes of this research. All the Na was removed from the samples, and very high percentages of the sought-after trace elements passed through. These other elements, however, are not believed to interact chemically with the resin.

It was concluded from testing that the presence in the resin of some long-lived elements after elution was due to the limited sample digestion techniques used. The digestion employed was limited because of Se, a sought-after volatile element, with the result that high temperatures could not be employed. Thus, the non-dissolved parts of the sample are not able to pass through the resin, and could cause quantitative underestimations. The resin has been employed in the removal of Na from seawater (Nyarku and Chatt, 1982), glass (Gills et al., 1970), rock samples (Ralston and Sato, 1971), potatoes[2] (Torok et al.,1973), standard reference materials (Guinn et al.,1977), whole blood (Ward et al., 1979) , and serum (Woittiez and Das, 1982). Krishnan and Crapper (1975) tested the retention of the resin for several trace elements, for five different concentrations of sulfuric, perchloric, nitric and hydrochloric acid mediums. The best results came from 12N

---

[2] This 6-page paper was fully translated from German by the author for this study. Copies are available at the UFTR Radiochemistry laboratory.

HCl medium, in which sodium was quantitatively (>99.9%) retained while many other elements were quantitatively eluted.

Sodium removal prior to separation is an alternate procedure, attempted in serum by Woittiez and Das (1982), but it has three setbacks:

1. A very clean laboratory is necessary, to keep samples free from trace contaminants prior to irradiation. Laboratory apparatus and even the resin should be assigned special cleaning protocols. Clean laboratories are sophisticated and expensive facilities, requiring trainned personnel for their operation and maintenance.
2. The samples result in wet and highly acidic mediums. They require freeze-drying, which is not an advised alternative for low pH samples, or ashing, if no volatile elements are under consideration, or even quartz tube vials to contain the wet samples during irradiation.
3. The HAP resin leaches considerable amounts of Sb into the samples. After activation $^{124}Sb$, with 60.2 days half-life, becomes a major interference which is very difficult to control. Nyarku and Chatt (1982) made several attempts to reduce this interference, with limited success.

Post Irradiation Procedures

After elution, as described in Appendix B, the samples were counted. The practical impossibility of having the UFTR at 100 kW power for 28 consecutive hrs determined the following irradiation schedule, for each set of samples:

day 1: 9:30 AM - 5:30 PM
day 2: 8:00 AM - 6:00 PM
day 3: 8:00 AM - 6:00 PM

On the fourth day the samples were retrieved from the reactor and taken to the "hot cell" in the UFTR Radiochemistry Laboratory. They were immediately processed, in order to be ready for counting at 1:00 PM of the 5th day. This procedure usually required overnight monitoring to check for adequate digestion of the sample. Problems involved with sample digestion are discussed in the next section.

Since several samples were to be irradiated at the same time and under a discontinuous schedule, a special procedure for counting and positioning of samples was designed. This procedure maintained the integrity of the comparator methodology, as described in Appendix A.

Copper, K and Br peaks were accumulated within 60 hrs after the end of each irradiation.

The accumulated counting spectra were analyzed by a computer-based analyzer system, a Tracor-Northern (TN-11) analyzer coupled to a intrinsic Ge detector (Ortec), and

equipped with a floppy-disk-drive for storage of up to 19 spectra on one 8" disk. Two ADC's acquire from two detectors simultaneously. Back-up copy disks were always produced to avoid accidental loss of data.

Approximately 3 weeks later, the same samples were counted for the longer lived elements Zn, Cr, Fe, Se and Co.

Analysis was performed as before for each of the 84 samples and 24 standards. A total of 8 long-time irradiations were performed in the UFTR within a period of 4 months. The final results are plotted in Chapter IV.

## Testing of Alternative Procedures

Documented research on HAP applications on neutron activation of high-sodium-content-materials shows nitric and hydrochloric acids as the most preferred acids for sample digestion and resin elution. Krishnan and Crapper (1975) have made extensive evaluation of several acids. When highly concentrated, hydrochloric and nitric acid mediums allow more than 99.9% sample sodium retention. Other acids studied were sulfuric and perchloric acids. The literature is not explicit, however, on how the dry serum sample should be digested. Acids alone, even when highly concentrated, will take many hours for partial sample digestion. Heat was used in this study because it proved to promote quick digestion, turning the sample into a clear brownish liquid. However, elevated temperatures jeopardized the stability of

Se in the sample. The boiling point for this element is 685°C (CRC manual 1983) and 60°C was the digestion temperature chosen for this analysis. This temperature was expected not to produce significant loss of Se even in eventual hot spots that might eventually form inside the liquid. Infrared lamps were used for this purpose. Their positions and distance were determined through measurements using a thermometer placed inside an acid blank.

Hydrochloric acid showed better digestion properties than nitric acid for serum samples. A precipitate was formed when nitric acid was used, which would sometimes stick to the test tube walls, not offering conditions for adequate quantitative removal. This precipitate was in the form of yellowish lumps of low density material and 1 to 2 mm in diameter.

The use of 12N HCl acid produces the same type of sticking lumps but in much lower amounts and smaller size. Serum samples can be digested well enough to not show any of these irregularities. There is an interval of time during digestion in which the sample shows itself free of any lumps, which are probably due to the formation of some complexes, in a process catalized by the applied heat. Lumps can be avoided if the heat is turned off as soon as the sample can be, by visual inspection, considered digested. High levels of radiation exposure, however, will limit the presence of the experimenter to a few minutes each

hour. The existence of these lumps can cause accumulation of the sample on top of the resin. The two main effects of this are:

1. The non-digested part of the sample will not pass through the resin, ultimately resulting in quantitative underestimation of concentrations.
2. These lumps would sometimes clog the resin. Heating of the column with infrared lamps seemed to solve the problem by thermal convection of the sample solution, but not without producing bubbles in the inner resin due to boiling of HCl. These bubbles cause loss of retention power for the resin. Higher amounts of it are then necessary. As well new studies are necessary in order to evaluate its behaviour under different temperatures.

Paper filters were used in order to control the problems produced by limited sample digestion. They were placed inside the funnels and on top of the resin. Any lumps would be kept on the filter, which would, after the first count for short-lived elements, be placed inside the 75 ml beakers for decay and later yielding of concentration values for the longer-lived elements. The filters pass to contain high amounts of radioactive Na, and for this reason they cannot be placed in the beaker for the first count. Some underestimation of Cu, K and Br concentrations will result, of course, since many of these atoms stay in the filter.

These amounts were not considered to be significant when compared to sample levels.

Biological samples produce large peaks due to K and Br. Their half-lives are 12.36 hrs for $^{42}K$ and 35.3 hrs for $^{82}Br$, respectively. The identification of any other trace elements turns out to be very difficult during the first week after irradiation. Only Cu, K and Br can be effectively identified and quantitated. The filters were throughly washed with 12N HCl while still in the funnel columns. No specific measurements were made here, due to the presence of high Na interfering activities. The sought-after longer-lived Co, Se, Fe and Zn possess half-lives in the order of months or longer, namely,

Co: 5.27 years ($^{60}Co$)

Se: 120 days ($^{75}Se$)

Fe: 44.6 days ($^{59}Fe$)

Zn: 243.8 days ($^{65}Zn$)

One element, Cr, whose activation product $^{51}Cr$ has a 27.7 day half-life, could not be detected after 3 weeks of decay. It might be possible to detect Cr if Br and K were removed from the sample.

Woittiez and Das (1982) have designed a procedure that allows elimination of Na, Br and Cl from serum. It involves the mineralization of 1 ml of serum with 2 ml 14M nitric acid in a Teflon-lined pressure vessel at 140°C for 2 hrs. After mineralization, the sample is evaporated to

dryness (removal of Cl, Br and I). The residue is then redissolved in 1 ml nitric acid. The high temperature step is intended to mineralize the sample, making the Br and Cl atoms free to volatilize under the heat lamp. The same procedure was attempted in this study, without the high temperature mineralization step because of the volatility of Se. Bromine proved to suffer no evaporation of any sort in this case. After the evaporation step the sample should have its pH lowered and then be eluted through the resin for Na removal. Torok et al. (1973) separated Na, K and P with the help of resins, in an automatic apparatus for continuous digestion, elution and counting of biological activated materials. They showed that the resin would work well in a practical application when control existed over a series of characteristics like amount of resin, grain size, sample pH and elution speed.

## Radiation Exposure Levels

The design of experiments of such type has to include radiation safety considerations. Eight irradiations, of 3 days each, were performed for 12 samples at a time. Even after 1 day decay inside the reactor, the sample assembly described in Appendix A showed exposures of 350 mR/hr at 1 foot. The assemblies were shielded and then rushed to the hot cell, held at as great a distance as possible from the body. Less than 2 mrem whole body dose were received during this transportation step for each batch of 12 samples.

Once inside the hot cell, the samples were unpacked and disassembled through the use of mechanical arms. The columns were pre-assembled, so that the experimenter could do the work faster and without staying in the laboratory longer than necessary. All glassware was ready, acid bottles full and infrared lamps on and positioned. Any activity subjected to advanced preparation was performed as such.

The vials were individually taken to the fume hood in 12 carefully executed transferring procedures.

Their contents were qualitatively[3] transferred to test tubes arranged in a way to receive infrared light which would bring their contents up to 60 °C. Empty vials offered exposures in the order of 10 mR/hr at 1 foot distance. Their contents were responsible, in average, for 7 mR/hr exposure at 1 foot. When ready for digestion, the whole assembly showed exposures in the range of 100 mR/hr at 1 foot.

After adding few ml of acid to it, the experimenter left the laboratory for the following hour, for this is the approximate length of time necessary for the digestion to

---

[3] The powder serum samples were transferred after irradiation to test tubes for acid digestion. The high radiation levels made quantitative transfers prohibitive because of time exposure considerations. In order to solve this problem, the full vials had their weight recorded before irradiation, and were reweighed after decay (one month later) to estimate vial plus sample remains in it. Subtraction of the latter values from the first ones yields the weight of the powder sample transferred to the test tubes for immediate acid digestion.

start taking place. At this point, radiation absorbed doses were as shown in Figure 3.1 for the accumulated body distribution doses after batches number 1 (top) and number 8 (bottom). Absorbed doses are much lower for the last batch, suggesting that the radiation safety considerations which were progressively added to the experiment protocol resulted efficient and appropriate.

The fume hood was equipped with lead shielding and negative air pressure, due to the expected high levels of radiation and acid fumes. A HEPA filter was placed in the air outlet of the fume hood.

The funnels containing the resin were placed behind heavy shielding since during elution they were radioactive enough to interfere with any sample being counted on the detector. The experimenter inspected the set-up every hour. Heat lamps were turned off if the samples looked digested.

Special attention was always given to the resin, which becomes radioactive from the moment of elution by holding $^{24}Na$ atoms. It was thereon treated as radioactive material possessing a half-life of 15 hrs, as determined by $^{24}Na$. Even after elution, when sample activity drops to very low values, the samples were kept away and shielded from the detectors since they are very sensitive to gamma radiation.

Background evaluations were done for each set of measurements, in order to identify any possible new sources of radiation in or outside the laboratory.

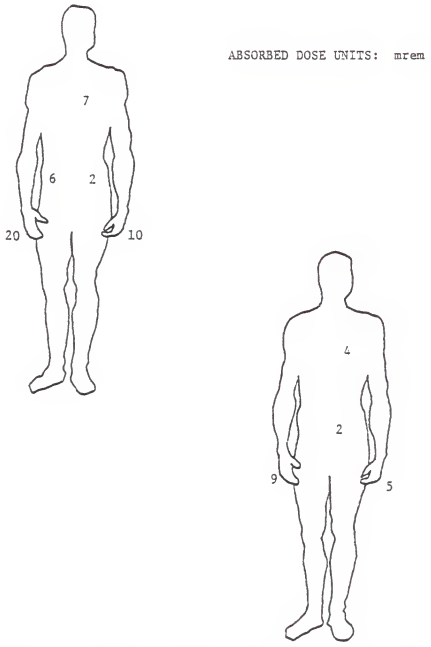



Figure 3.1. Accumulated body distribution doses for batches number 1 (top) and number 8 (bottom).

# CHAPTER IV
# RESULTS AND DISCUSSION

## Results

Results of the serum analysis for this study follow in graphical form in Figures 4.1--4.21. Trace element concentrations are plotted against time. The $T^0$ time is always the bone marrow transplant date for each patient. Results for the controls were plotted without following any particular rule with regard to time.

The coding numbers are patient's identification numbers at Shands Hospital. The graphs show trace concentrations for acute myelogenous leukemia, chronic myelogenous leukemia, acute lymphoblastic leukemia, aplastic anemia, clinically healthy (control) subjects and ror two other disease conditions also requiring bone marrow transplantation for their treatment.

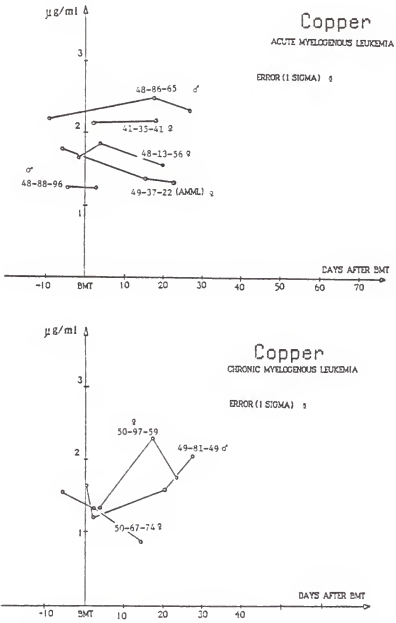



Figure 4.1. Serum Cu concentrations for acute myelogenous leukemia and chronic myelogenous leukemia patients.

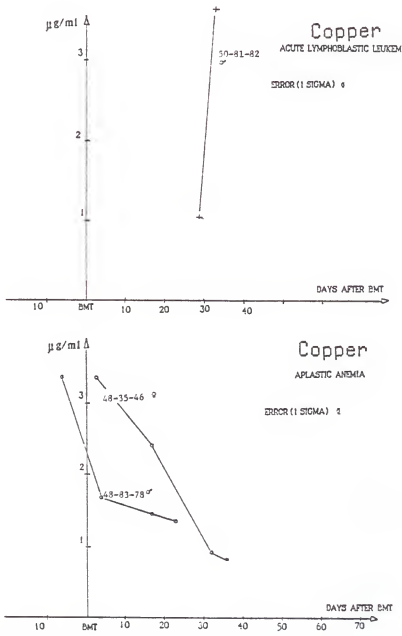
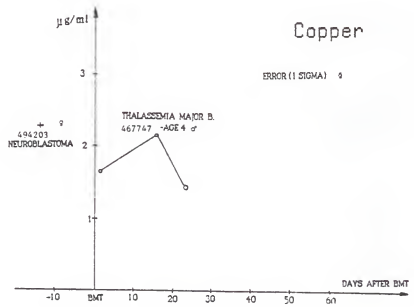



Figure 4.2. Serum Cu concentrations for acute lymphoblastic leukemia and aplastic anemia patients.



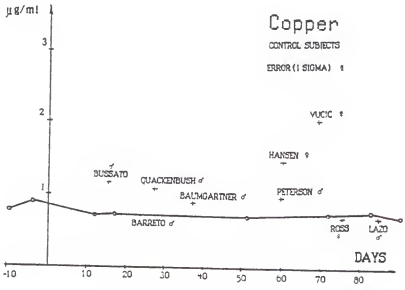




Figure 4.3. Serum Cu concentrations for other disease conditions and for 9 control subjects.

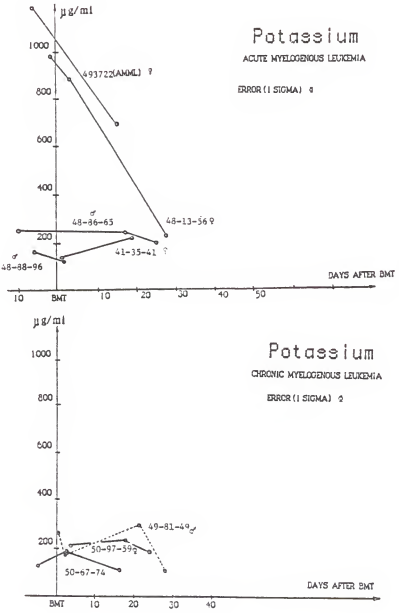



Figure 4.4. Serum K concentrations for acute myelogenous leukemia and chronic myelogenous leukemia patients.

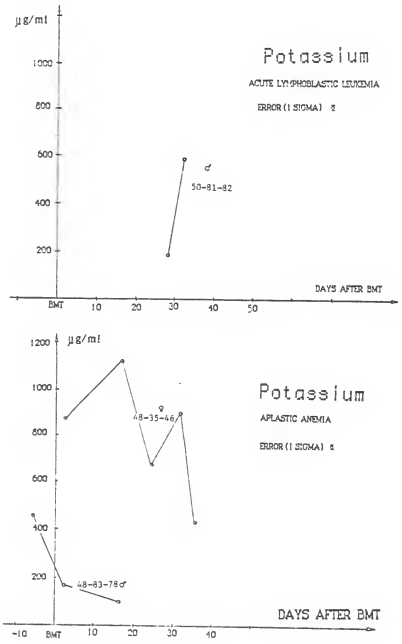



Figure 4.5. Serum K concentrations for acute lymphoblastic leukemia and aplastic anemia patients.

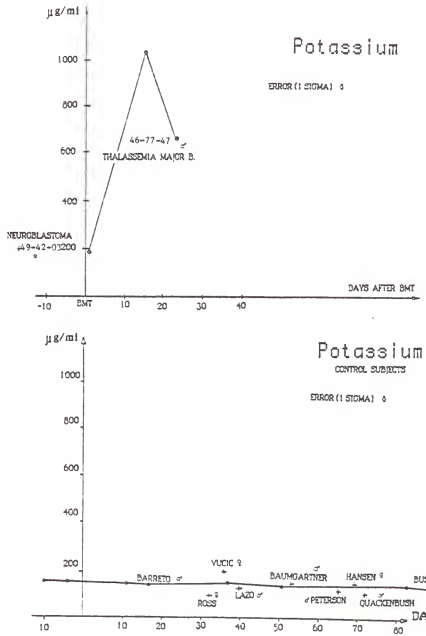



Figure 4.6. Serum K concentrations for other disease conditions and for 9 control subjects.

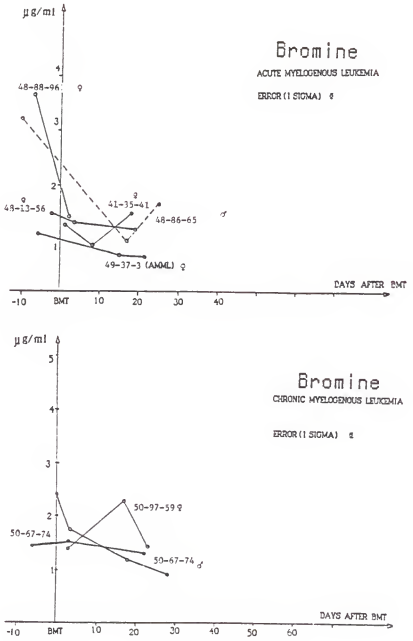



Figure 4.7. Serum Br concentrations for acute myelogenous leukemia and chronic myelogenous leukemia patients.

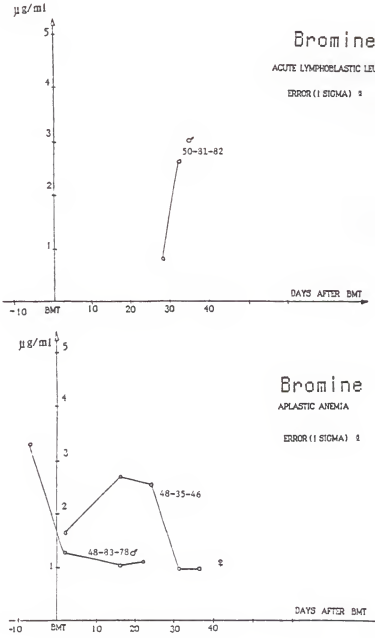



Figure 4.8. Serum Br concentrations for acute lymphoblastic leukemia and aplastic anemia patients.

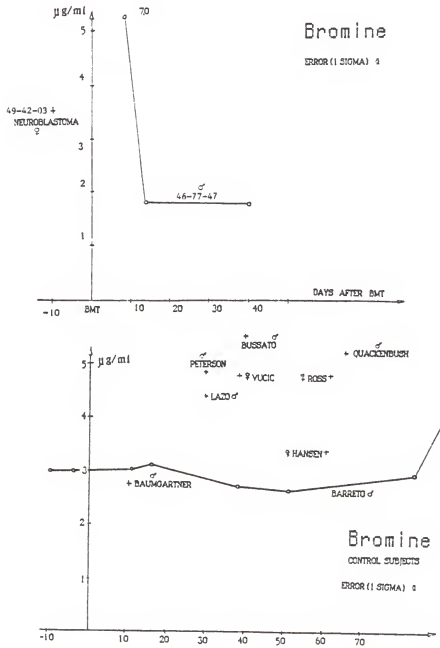



Figure 4.9. Serum Br concentrations for other disease conditions and for 9 control subjects.

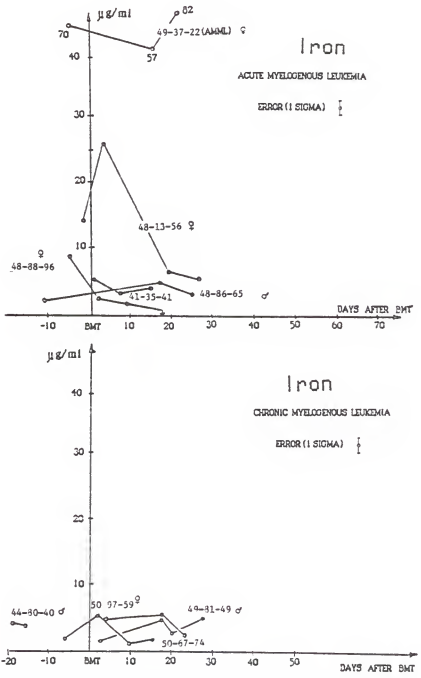



Figure 4.10. Serum Fe concentrations for acute myelogenous leukemia and chronic myelogenous leukemia patients.

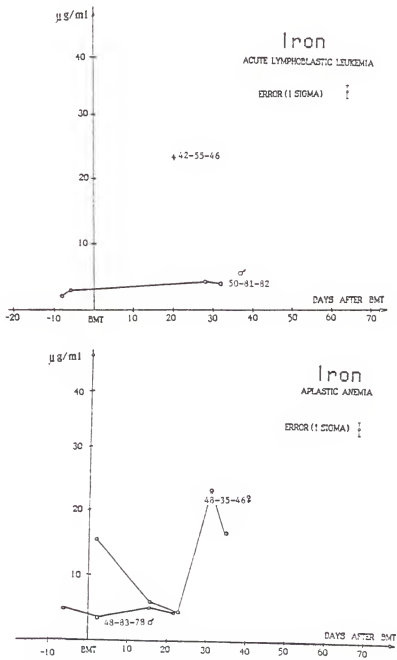
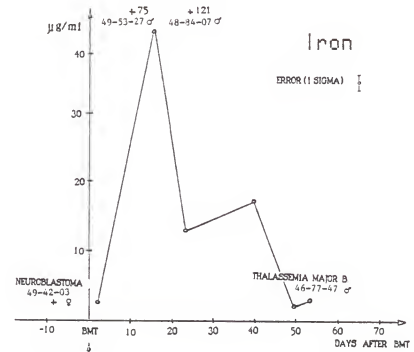



Figure 4.11. Serum Fe concentrations for acute lymphoblastic leukemia and aplastic anemia patients.



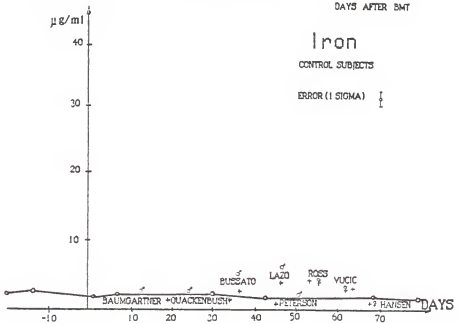




Figure 4.12. Serum Fe concentrations for other disease conditions and for 9 control subjects.

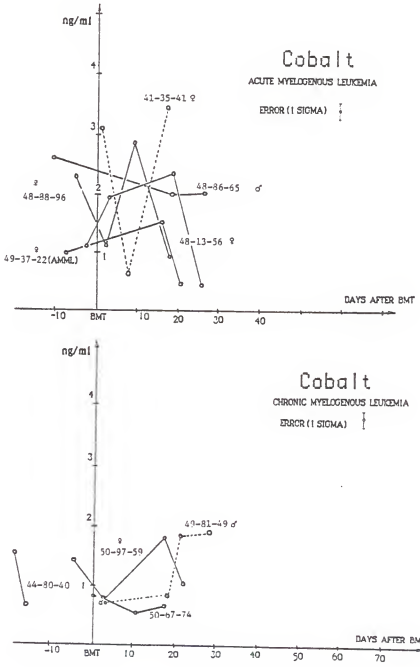
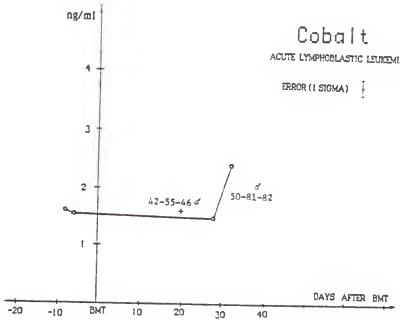



Figure 4.13. Serum Co concentrations for acute myelogenous leukemia and chronic myelogenous leukemia patients.



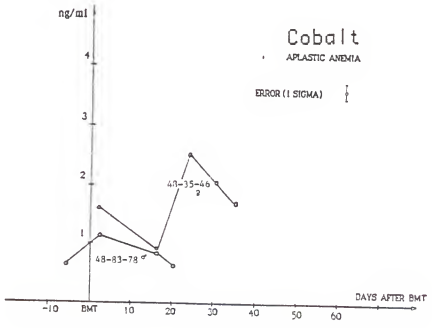




Figure 4.14. Serum Co concentrations for acute lymphoblastic leukemia and aplastic anemia patients.

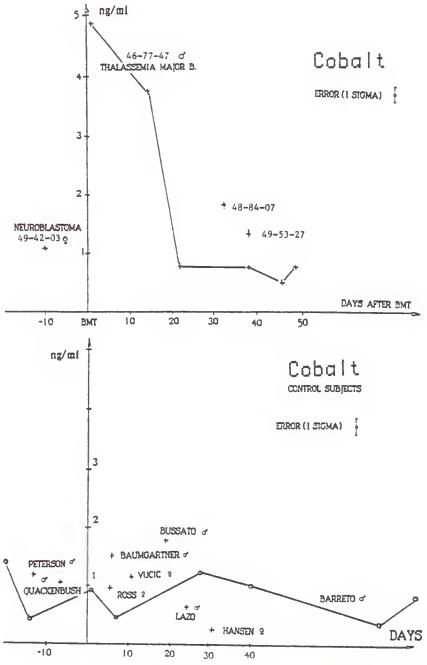



Figure 4.15. Serum Co concentrations for other disease conditions and for 9 control subjects.

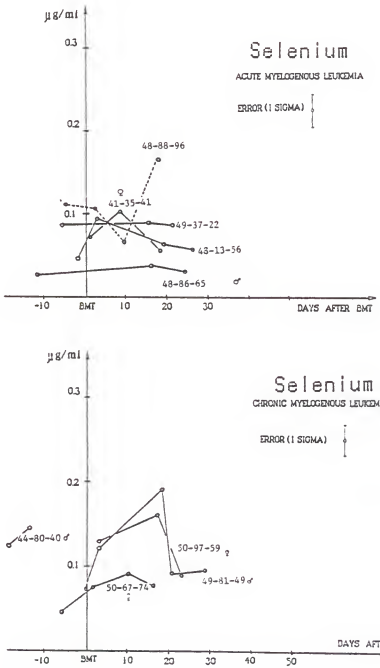



Figure 4.16. Serum Se concentrations for acute myelogenous leukemia and caronic myelogenous leukemia patients.

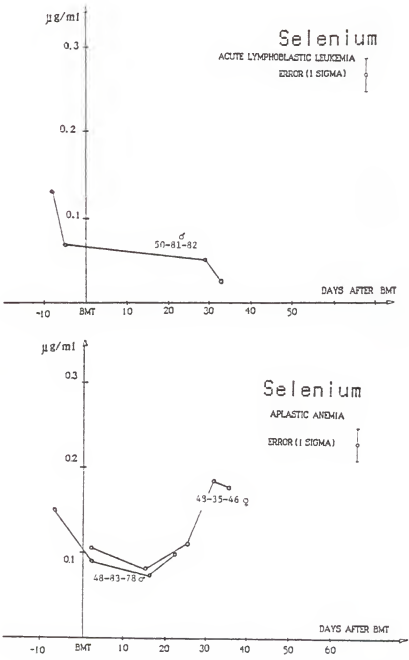



Figure 4.17. Serum Se concentrations for acute lymphoblastic leukemia and aplastic anemia patients.

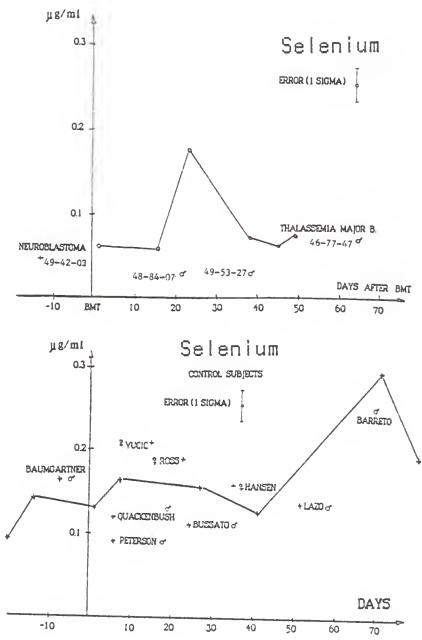



Figure 4.18. Serum Se concentrations for other disease conditions and for 9 control subjects.

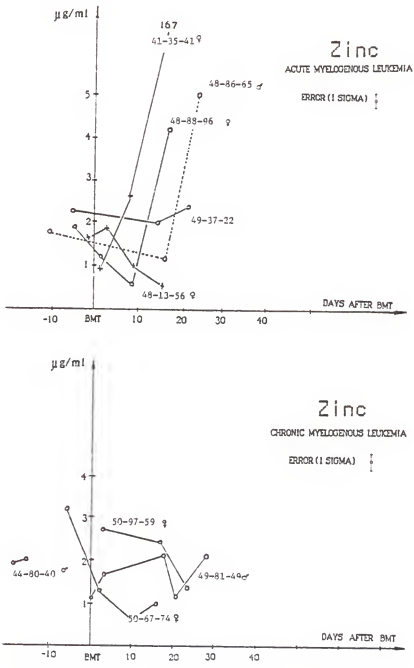



Figure 4.19. Serum Zn concentrations for acute myelogenous leukemia and chronic myelogenous leukemia patients.

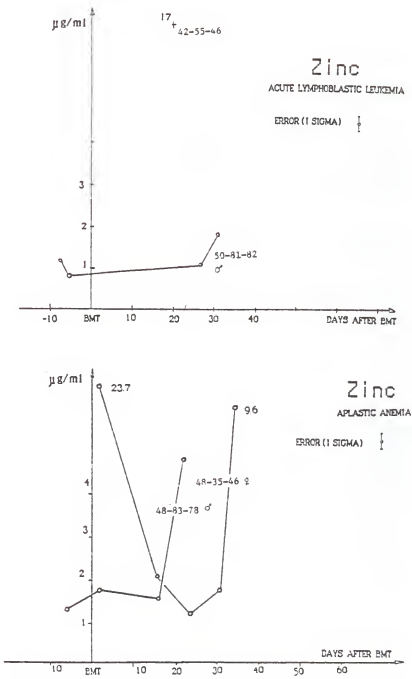



Figure 4.20. Serum Zn concentrations for acute lymphoblastic leukemia and aplastic anemia patients.

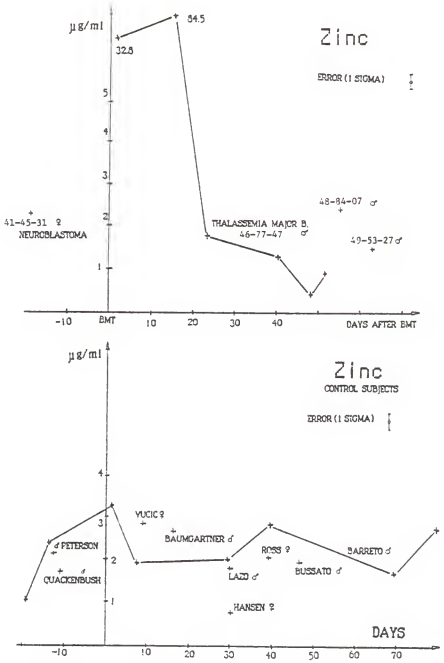



Figure 4.21. Serum Zn concentrations for other disease conditions and for 9 control subjects.

Figures 4.22--4.25 show average concentration values for leukemia patients undergoing bone marrow transplant. They represent averages of all leukemia values plotted in Figures 4.1--4.21. Control averages were also plotted for comparative purposes. The arrows indicate confidence intervals (1 sigma). Aplastic anemia averages were not plotted because only few patients had such condition.

A third value is listed in each figure, for comparison with other serum studies. Such value was extracted from the literature and reflects, in the author's opinion, accurate values for normals in serum analysis. Such opinion is based on the attention dedicated by the experimenter to sampling, sample preparation, counting and analysis procedures for the serum samples.

The sources of the literature values are listed in the section Trace Element Studies for Serum of Chapter I.

## Discussion

This study has accomplished three main objectives. First, it demonstrated that neutron activation analysis (NAA) can be used with biological materials using the University of Florida Training Reactor.

Researchers usually avoid NAA of such materials because of their high content of readily-activated interfering Na by using other well-established methods such as atomic absorption spectroscopy for trace element determination.

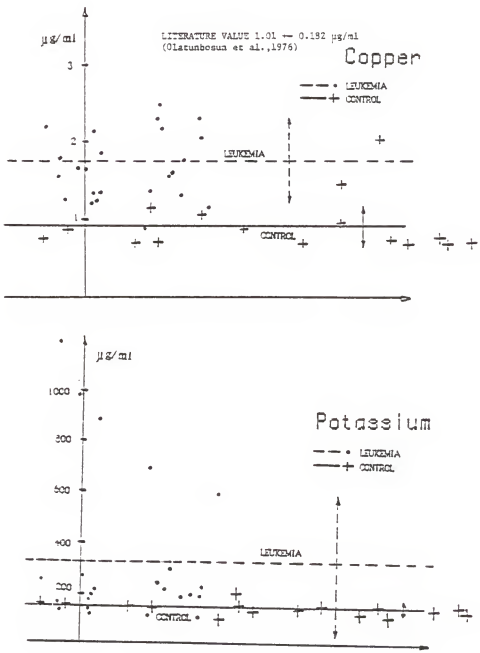



Figure 4.22. Serum Cu and K average values for leukemia, controls and literature value.

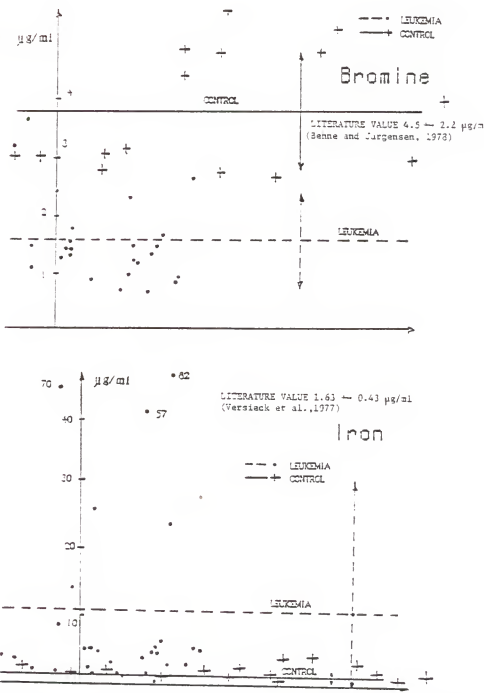



Figure 4.23. Serum Br and Fe average values for leukemia, controls and literature value.

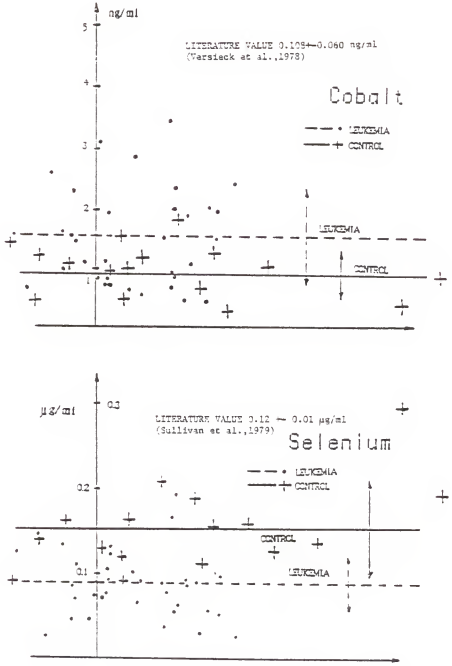



Figure 4.24. Serum Co and Se average values for leukemia, controls and literature value.

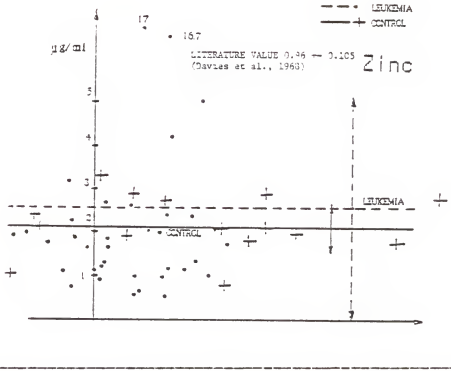



Figure 4.25. Serum Zn average values for leukemia, controls and literature value.

Neutron activation analysis presents some advantages over other techniques, for it can do simultaneous determination in minute samples while attaining high precision and accuracy. Such characteristics are important in disease conditions where there may be synergistic effects between concentration levels in samples of small size.

Secondly, this study demonstrated the usefulness of the University of Florida Training Reactor (UFTR)--one of the two research reactors in the southeast for NAA of biological samples. The unavailability of a high thermal neutron flux allied with the interrupted operation during night hours

makes the UFTR virtually unfit for single long time irradiations as is possible in some modern research reactors. The reactor can operate overnight only if substantial funds are provided.

However, proper design and application of an irradiation protocol based on the comparator method can successfully overcome such limitations.

Such a protocol was developed during this study and allows longer irradiations to be made in daily steps (in order to compensate for the relatively low flux) and makes evident the UFTR's capability for short (through its pneumatic transfer system) and long irradiation of samples of most matrixes, including the problematic biological ones, 12 or more at one time.

Serum samples occupy a unique position among others of biological matrix since they possess high Na and low trace element content. The capability for total Na removal from serum samples suggests the feasibility of analysis of other biological samples.

Sodium removal was performed through the use of hydroxy antimony pentoxide (HAP) resin which proved effective in spite of literature evidence that it presented inadequate characteristics for analytical work (Berry, 1979). It was show in this study that the resin can function efficiently even in the limited conditions of mineralization applied to these serum samples. Extreme care was necessary for the

resin conditioning, column assembly and elution steps in order to enhance the positive characteristics of the resin. Elements Cu, K, Br, Co, Fe and Zn proved to eluate through the resin while Na was fully adsorbed. However, only about 90% of the Se atoms pass through under the conditions used in this study. The low boiling point of Se did not allow for adequate sample mineralization, as suggested by Woittiez and Das (1982). Future research should attempt to find variations for the method which would allow Se to be integrally recovered.

Thirdly, the extreme care taken during sampling and analysis proved to be effective in avoiding extraneous additions of trace elements to the samples. The results for the 17 3 cc serum samples taken from clinically healthy subjects are well inside the range recently obtained by other researchers.

The analysis of bovine liver SRM's (Standard Reference Materials produced by the National Bureau of Standards) showed acceptable accuracy in the concentration determinations of Fe, Cu, Co and K (Appendix C).

The results obtained from the analysis of 67 3 cc serum samples from bone marrow transplant patients demonstrate, in some situations, that either the <u>disease condition</u> or the <u>hyperalimentation treatment</u> (the latter designed to avoid malnutrition of such patients during their ceasing of oral caloric intake) is causing extremely high levels of some

elements in serum, possibly edging toxic concentration levels.

Figure 4.22 shows that the serum Cu concentration average is higher in leukemia patients than in the control group. Potassium levels tend to be essentially the same, except for few values which are extremely high.

Figure 4.23 suggests that Br values are consistently higher in the control group than in the leukemia patients group. Normal levels are seen for Fe even for the patient's group, except for 1 patient whose levels are 100 times higher than the control average.

Figure 4.24 shows that Co levels vary widely, more in the patient group than in the control group. Selenium concentrations are lower in leukemia patients' serum than in controls' serum. Figure 4.25 shows high variability in Zn levels, probably due to syringe Zn contamination. Zinc reached a 10-fold level for 2 leukemia patients as compared with average leukemia patients' serum levels.

In spite of the diversity of serum levels detected, it was concluded that meaningful clinical conclusions cannot be drawn from this data alone.

Statistical treatment of the results proved to be inadequate in the scope of this study because of two main aspects:

1. Several disease conditions were involved. There were few patients sampled within each condition and each

condition had potentially different influence on the behavior of trace concentrations during I.V. alimentation.

2. Inputs were too numerous and varied. Medication, blood transfusions, infections, feeding by mouth, the stress associated with the disease, TPN solutions and no "window" periods during hyperalimentation are a few examples. For this study, however, with collaboration from the Department of Pharmacy Services of Shands Hospital, all I.V. solutions were prepared following standard procedures, avoiding Al alloy needles during its preparation and as many patients as possible were placed under the same schedule of I.V. feeding, for ease of sampling and maintenance of parameters throughout the research period.
3. The circadian rhythm was also considered to be a source of variability in the data. It involves recognition of acrophases as a result of complex cellular mechanisms that are influenced by light/dark, time of day/year, hormonal regulation, etc. As a result, some elements suffer large daily variations in concentration.

It was observed that the levels for the controls were usually well grouped, indicating reproducibility in the analysis technique employed. This fact can be noted with

higher certainty for the points representing the control subject who was continuously sampled weekly for 3 months.

Such consideration in itself indicates precision in the methodology employed. It should be observed that the control variation observed for other elements like Fe, Co, Zn and Se in control samples might be due to the scale factor used when plotting the graphs. The ordinate-axis was usually only high enough to accommodate all values within each set of figures for each element.

Future research on effects of medication or any other factors mentioned above should concentrate on animal research where no strict protocols have to be observed, making possible daily sampling and close follow-up of acute or chronic additions of medication. Animal fluids could be then correlated with tissue stores, effects would be isolated and a model for trace element behavior in health and disease would be ultimately assembled.

In perspective one should consider the present knowledge on trace elements and their importance in health and disease. In summary, one can tell for some elements whether deficiency or toxicity is occurring, but the quantitative limits at which these two important limits occur is barely known. Similarly, the body tissue in which monitoring should occur for a certain trace element is a matter of discussion, for the ideal tissues sometimes can be sampled only through surgery. One reason for such lack of

knowledge is due to the technical difficulties in analysis, since levels although important are usually extremely low.

A Na-free sample environment, free of contamination and quantitative losses, makes the high accuracy and precision of neutron activation available to clinical evaluation of body samples in health and disease. As a natural extension of its virtually infinite capabilities, the developed technique allows the assessment of trace elements in animal and marine research, monitoring of toxic levels in foods, environmental surveillance, plant metabolism studies, criminalystic and any other which may involve the detection of elements in biological samples, i.e., samples of high Na content.

## CHAPTER V
## CONCLUSIONS

1. It was shown that the University of Florida Training Reactor can be used in long irradiations for trace element determination in clinical samples.
2. The limitations of discontinued UFTR operation during night hours and of relatively low thermal flux ($1*10^{12}$ neutrons/$cm^2$s) of the UFTR can be overcome through the use of long irradiations in a number of daily steps under a comparative, sample and standard methodology.
3. Serum concentrations of Cu, K, Br and Fe were determined with errors of less than 5 %. Elements Co, Zn and Se had their concentration evaluated in serum with errors of less than 15 %. The accuracy of the results was verified by the use of National Bureau of Standards' Standard Reference Materials.
4. Hydroxy antimony pentoxide was succesfully employed for Na removal in samples in spite of recent discouraging literature reports.
5. It was shown that only about 90 % of serum Se will pass through the HAP resin under the conditions used in this experiment.

6. Selenium, K, Cu, Co, Fe, Zn and Br can be simultaneously determined in small-size serum samples in clinical studies.
7. Modern sampling considerations and techniques were employed in order to avoid sample contamination before analysis and tests showed only contamination was due to Zn leached from plastic syringes.
8. Results in the patient groups are quite different from the control values. However, no conclusive clinical conclusions can be made from the data in this study.

   One of two possibilities exist. Either the hyperalimentation fluid is not appropriate for use and is producing extremely high and possibly toxic concentration levels or serum is not a valid indication of the elemental level. It is important that this be explored further.
9. Patient's serum presents very complex behaviour regarding trace elements due to treatment conditions. Studies of this kind should concentrate on careful experimental design in order to build a model for trace elements in body tissues and fluids.
10. Animal research is recommended for nutrition studies regarding trace elements with applications in disease conditions. Such studies can employ the technique developed in this study.

## APPENDIX A
## THE COMPARATOR METHOD APPLIED TO SERUM ANALYSIS

One of the main objects of this study was to make it possible for the University of Florida Training Reactor to be used in accurate and precise determination of essential trace elements in biological and clinical samples. The UFTR presents a relatively low neutron flux when compared to modern research reactors. Also, the impracticability of having it operating continuously for several days makes it limited for precise analysis of longer lived activation products, which is the case for the trace elements under consideration in this study.

These limitations can be, as demonstrated in this study, overcome with the use of longer irradiation times and a properly applied irradiation and counting protocol known as comparator method.

Neutron activation analysis (NAA) can be performed under one of two basic protocols: instrumental NAA, in which no chemical treatment is applied to the sample, neither before nor after irradiation, and chemical NAA, which involves selective chemical separations for the sought-after elements. Under each one of these protocols, the gamma-spectrum obtained by counting the post irradiated samples

will need mathematical interpretation. Two are the usual choices here:

1. A classical instrumental approach, in which the "activated sample" formula is used. In this approach, several empirical values are necessary. Some of these values must result from careful measurement, i.e. the isotopic abundance in nature which depends on geographic location. The formula is given below. It requires exact knowledge of thermal neutron flux at the position of irradiation inside the reactor, cross-sections, isotopic abundance in nature, irradiation time, dead-time for the detector, and other not so evident values which must be taken into account.

   The measured activity R which is induced in an element after an irradiation time t (case of simple activation) is given by

$$R(t) = w.z.a.N.c.F \frac{1-\exp(-0.693*t / T1/2)}{A}$$

where:

F = thermal neutron flux;
R = measured activity in cps;
w = weight of the irradiated element in grams;
z = efficiency of the detector;
a = abundance of the activated nuclide;
A = atomic weight of the irradiated element;
N = Avogadro's number;
T1/2 = half-life of the radionuclide formed;
t = irradiation time; and
c = isotopic activation thermal cross-section.

2. The comparator method allows determination of concentrations in the sample by comparing them to the ones obtained from a well known standard, which received the same irradiation, decay and counting treatment as the sample. The rationale comes from the classic formula presented above. For a certain element, writing the formula for the unknown sample and again for the standard, the ratio between the two will cancel out values like cross-section, isotopic abundance and neutron flux.

Standards normally have to be produced for the purpose of quantitative analysis. Carefully weighed amounts of the sought-after elements are irradiated under the same conditions as the sample. The same position inside the reactor, irradiation time length, decay time, counting time, counting geometry and the detector geometry are the conditions for the successful use of the comparator method. Many of these requirements have been dismissed in analysis performed with the use of modern computerized analyzers, since the software available with these systems usually has the capability of correcting any deviations from the basic protocol.

The Tracor Northern-11 analyzer software requires that the mentioned protocol be followed with no variations. The calculation of trace element concentrations results then in a simple formula:

$$\frac{\text{Conc in sample}}{\text{Mass of sample}} = \frac{\text{Conc in standard}}{\text{Mass of standard}}$$

The simplicity of the formula hides the several requirements stated above, which are sometimes difficult to meet, as exemplified in this study.

Twelve of the serum samples[1] were simultaneously irradiated in 3 steps, i.e., 3 days, totalling 28 hrs of irradiation. The long irradiation time was justified by the necessity of activation of the long lived elements Zn, Co, Cr, Fe and Se.

The opportunities are not many in which the reactor is available for 3 consecutive days. For this, a high number of samples (12) had to be irradiated at one single time. New problems became evident as Cu, K, and Br would be the first elements to be determined. They should be counted within 2 or 3 days, for their half-lives are relatively short, ranging from 12.4 to 35.3 hrs.

Very high radiation doses and the fact that the samples would be counted practically right after irradiation of 28 hrs turned into problems of both safety and analytical considerations. Calculations alone would not evaluate the concentrations correctly, if the TN-11 software were to be

---

[1] Only twelve samples could fit in the central and east vertical ports of the UFTR at the same time.

used. Therefore, the same decay and counting procedure would have to be followed for samples placed in one same irradiation position. Such problem arose the necessity for the use of the comparator method.

Since the flux could vary vertically as well as horizontally, 12 positions were assigned by numbers from 1 to 12 inside the reactor. Nine of them in the central vertical port (CVP) and 3 in the east vertical port (EVP) which is smaller. The bottom of the CVP was conceptually divided into 3 levels, each level containing 3 samples. Figure A.1 describes the assembly and shows the aluminum cup in which the samples are positioned.

The cup is lowered into a positioning device which orients it inside the reactor. This position assurance device allows precise positioning of the 9 samples inside the CVP. The flux in the EVP varies vertically, as well as horizontally. The 3 samples in the EVP were placed one atop the other for fitting, and positions 10, 11 and 12 were assigned to them. Any horizontal flux gradients should have their effect balanced within each sample. One side of it would be exposed to a higher flux, and so would the standards and all other samples, any adverse effects being then compensated for. It became evident at the end of this study that the horizontal variations inside the CVP were not significant for the purposes of neutron activation analysis. The positioning device, consisting basically of a long

aluminum tube, may have helped to homogenize the flux at the irradiation position.

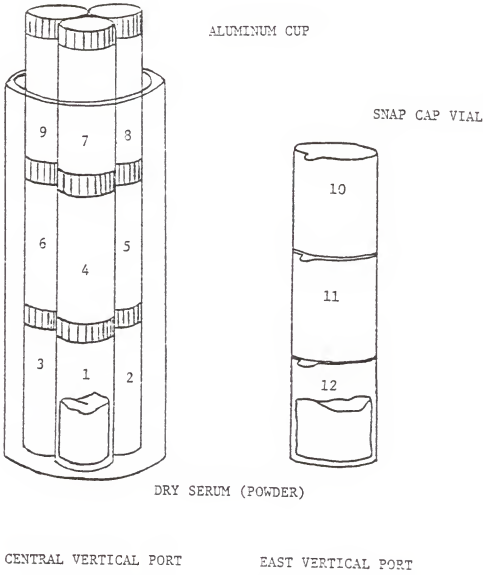



Figure A.1. Sample positioning inside irradiation aluminum cup.

After irradiation the samples were allowed to decay for 20 hrs inside the reactor. Very short lived radionuclides are responsible for very high initial radiation exposure rates. Twenty hours of decay would not significantly affect experimental procedures, but would bring working conditions down to safer levels. The radiation exposure rates involved are discussed in chapter II.

Samples were processed through the resin for Na removal and made to assume a 75 ml geometry in Pyrex(R) No. 1000 beakers. The samples would be ready for counting 40 hrs after the end of the irradiation period. The ones on the top of the assembly, placed at positions 7, 8, 9, and 10 would be counted first. Since the flux was expected to be lower for the higher levels, these samples would presumably have lower activities. The counting protocol was designed as shown below, each sample being counted for 2 hrs, detector live-time.

| position number | counted at * |
|---|---|
| 8 | day 2 - 1:10 PM |
| 10 | day 2 - 4:10 PM |
| 9 | day 2 - 7:10 PM |
| 7 | day 2 -10:10 PM |
| 4 | day 3 - 1:10 AM |
| 1 | day 3 - 4:10 AM |
| 3 | day 3 - 7:10 AM |
| 2 | day 3 -10:10 AM |
| 6 | day 3 - 1:10 PM |
| 5 | day 3 - 4:10 PM |
| 11 | day 3 - 7:10 PM |
| 12 | day 3 -10:10 PM |

*The time at the end of the irradiation (day 0 - 6:00 PM) is considered here as the reference.

The same procedure was followed for all other batches. Standards irradiated in position number 4, for example, would be used to calculate real concentrations for all the samples, in all 8 different batches, which were irradiated at position 4.

One set of 12 standards was prepared for the shorter-lived elements Cu, K and Br, and another set of 12 was equally produced for Fe, Co, Zn, Cr, and Se. The high deadtime observed for several samples lead to the counting of each short lived standard under two different geometries: 0 and 5 cm from the detector. Therefore, a sample irradiated in position 4 and showing too high activity at the time of counting would be moved to a 5 cm distance, and later have its concentration evaluated by comparing it to the standard irradiated at position 4 and counted at 5 cm distance.

After 3 weeks, the same samples were counted for 5 hrs each, all at 0 cm distance, for Se, Fe, Co, Cr and Zn. In this case, no special schedule was assigned for counting.

## APPENDIX B
## PREPARATION OF HAP FOR SODIUM REMOVAL FROM SERUM SAMPLES

The resin is known as HAP, and 500 grams of it were bought from Atomergic, Inc., NY, for $ 235.00. A list of research papers which describe HAP uses can be bought from the same company, which produces the resin under the name "Polyan HT".

Several are the points to observe when using HAP:

1. Only distilled-deionized water should be used for wet-conditioning, washing and assembling of the column. Tap water would saturate the resin with sodium ions, causing a loss in retention capacity.

   Two grams of resin are enough to hold sodium ions present in 0.4 g of dry serum.

2. The resin should be spread on a plastic sheet, one day prior to use, so that it could attain a higher degree of humidity. If not going to be used, it should be maintained in a dry place, in tight closed bottle.

3. Approximately half of the resin material is composed of very fine grains, which will cause clogging and slowing down, when not stopping the sample flow. This problem can be solved by repeatedly shaking a

mixture of resin and water, and discarding the supernatant (Gills et al., 1970).

4. The resultant resin can be kept for few days if immersed in water, in a closed container. It should not be let to dry if its future use is intended.

5. Funnels have worked well in maintaining the resin in a large length:diameter ratio, as desired for a column, and at the same time providing a large reservoir for non-processed sample.

   The funnels should be full of water before the slow dumping of the resin in it. This procedure would keep the settled resin free from air bubbles, which would eventually reduce the efficiency of the resin.

6. The resin needs then to be acid-conditioned. Five milliliters of 12N HCl should be run through the resin for this purpose.

7. A strong filter paper should be placed inside the funnel, on top of the resin, in order to keep non-dissolved sample material from reaching the resin and adsorbing to it. The filter paper will be recovered, cut into pieces and added to the sample when counting for long-lived radionuclides.

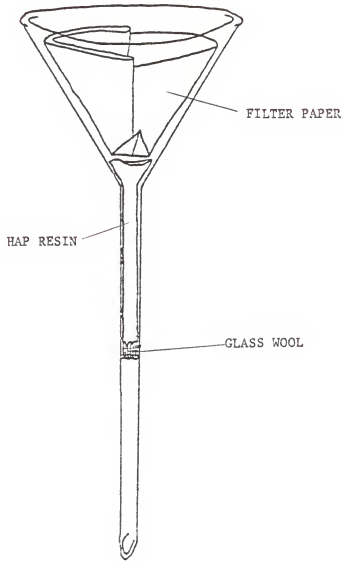



Figure B.1. The optimal assembling for the HAP resin in a funnel.

8. After the sample has passed through the resin, the resin should be washed with 12N HCl for any residual trace elements, until the standard volume for counting has been reached. Pyrex(R) no. 1000 beakers were used for this purpose, filled with diluted-processed sample to amount 75 ml. In average, each sample takes 5 hrs to complete the above mentioned elution.

9. The sample is then ready for counting. Since it is strongly acidic, the sample beaker should be covered with a thin plastic sheet, to protect the equipment and the experimenter from corrosive fumes and eventual spills during sample handling. The whole procedure should be performed inside a fume hood, well shielded for radiation. Hot cells, with remote sample handling performed with the use of mechanical arms, are the safest way to work long time irradiated serum samples and their containers. However, when not provided with adequate ventilation, hot cells will show an internal build-up of acid fumes, in these procedures strong enough to damage their delicate mechanisms.

10. It should be remembered that the sample has low activity after step 8. The resin, however, at the time of the first counting period, will show exposures in the order of 30 mR/hr at a distance of 1 foot.

The spent resin which will contain radioactive sodium (T1/2 = 15 hrs) should be kept away and shielded from the sensitive germanium detectors. Background counts should be done whenever appropriate, to check for counting interferences produced by other radioactive materials in the laboratory.

11. The spent resin should be kept for decay and disposed through the Radiation Safety Office. Dumping of radioactive material in the laboratory sink could produce a difficult to remove source of background radiation.
12. Some important tests were performed with the resin. They are described in Chapter III.

## APPENDIX C
## PRECISION AND ACCURACY OF CONCENTRATION DETERMINATIONS

Only 4 samples of bovine liver National Bureau of Standards' Standard Reference Material (SRM) no. 1577 were used for evaluating precision and accuracy in this study. A blood serum standard is also available. However, no elemental determinations have been performed on serum SRM's by official laboratories, therefore no certified values are available as yet.

This small number of SRM's was due to limitations in the availability of the UFTR for the 3 consecutive days of irradiations. Irradiation positions were preferentially assigned to patient's serum samples.

SRM's underwent the same treatment applied to the serum samples (Appendix B). Mean concentration values found for Cu, Fe, Co and K in such standards were within one standard deviation of the reported means in the Certificate of Analysis issued for bovine liver by the NBS.

Bromine concentration in bovine liver is not listed by the NBS. However, a compilation made by Gladney (1980) lists 8.7 +- 1.7 ppm as its most probable value. Values obtained in this study for bovine liver's Br were within the above listed range.

Zinc and Se concentrations showed reasonable accuracy (within 3 sigma). The error in the Se values might be explained by the resin's characteristic of retention of Se atoms, as described in the section Irradiations of Chapter III.

LIST OF REFERENCES


Barrett A.J.. Bone Marrow Transplantation. The Practicioner 224, 1348 (1980) 1009-1015.

Behne D. and H. Jurgensen. Determinations of Trace Elements in Human Blood Serum and in the Standard Reference Material "Bovine Liver" by Instrumental Neutron Activation Analysis. Journal of Radioanalytical Chemistry 42 (1978) 447-453.

Berry D.L.. Evaluation of Some Procedures Relevant to the Determination of Trace Elemental Components in Biological Materials by Destructive Neutron Activation Analysis. Doctoral dissertation. University of Iowa. USDOE contract no. IST821. Ames Lab., Ames, IA, U.S.A. (Jan 1979) 232p.

Bertinchamps A. J., S. T. Miller and G. C. Cotzias. Interdependence of Routes Excreting Manganese. American Journal of Physiology 211, 1 (1966) 217-222.

Cotzias G.. Importance of Trace Substances in Environmental Health as Exemplified by Manganese. In: Proceedings of the First Annual Conf. on Trace Substances in Environmental Health. D. D. Hemphill, Ed., University of Missouri, Columbia (1967).

CRC manual 1983. Handbook of Chemistry and Physics. CRC: Cleveland, Ohio, (1983).

Davies I. J. T., M. Musa and T. L. Dormandy. Measurements of Plasma Zinc. Journal of Clinical Pathology 21, 3 (1968) 359-365.

Faulkner W.R.. The Trace Elements in Laboratory Medicine. Laboratory Management 21, 7 (July 1981) 23-34.

Gills T. E., W. F. Marlow and B. A. Thompson. Determination of Trace Elements in Glass by Activation Analysis Using Hydrated Antimony Pentoxide. Analytical Chemistry 42, 14 (1970) 1831-1833.

Girardi F. and E. Sabbioni. Selective Removal of Radio-Sodium from Neutron Activated Materials by Retention on Hydrated Antimony Pentoxide. Journal of Radioanalytical Chemistry 1 (1968) 169-178.

Gladney Ernest S.. Elemental Concentrations in NBS Biological and Environmental Standard Reference Materials. Analytica Chimica, Acta 118, 2 (1980) 385-396.

Glucksberg H., R. Storb, A. Fefer, C. D. Buckner, P. E. Neiman, R. A. Clift, K. G. Lerner and E. D. Thomas. Clinical Manifestation of Graft-Versus-Host Disease in Human Recipients of Marrow from HL-A Matched Sibling Donors. Transplantation 18, 4 (1974) 295-304.

Guinn V. P., E.R. Christensen, K. De Lancey, W. W. Wadman III, J. H. Reed, N. Hansen, A. A. Samra and V. J. Orphan. Neutron Activation Analysis Trace Element Studies in Connection with the Offshore Drilling for Oil. Nuclear Methods in Environmental and Energy Research, Conf. 771072, Univ. of Missouri, Columbia (1977) 303-311.

Kasperek K., G. V. Iyengar, J. Kien, H. Borberg, and L. E. Feinendegen. Elemental Composition of Platelets. Part III. Determination of Ag, Au, Cd, Co, Cr, Cs, Mo, Rb, Sb and Se in Normal Human Platelets by Neutron Activation Analysis. Clinical Chemistry 25, 4 (1979) 711-715.

Kostadinov K. and R. Djingova. Trace Element Analysis of Biological Materials by Thermal and Epithermal Neutron Activation Analysis. Journal of Radioanalytical Chemistry 63, 1 (1981) 5-12.

Krishnan S.S. and D. R. Crapper. Sodium Removal by Hydrated Antimony Pentoxide in Neutron Activation Analysis. Radiochemical Radioanalytical Letters 20, 4-5 (1975) 279-287.

Layton P.B., B. B. Gallucci and S. N. Aker. Nutritional Assessment of Allogeneic Bone Marrow Recipients. Cancer Nursing 4, 2 (April 1981) 127-135.

Maenhaut W., L. De Reu, H. A. Van Rinsvelt, G. S. Hoessler, J. W. Swanson and M. D. Williams. Trace Element Analysis of Biological Materials: Complementarity of PIXE and NAA. IEEE Transactions on Nuclear Science NS-28, 2 (1981) 1386-1391.

Mertz Walter. The Essential Trace Elements. Science 213, 4514 (18 September 1981) 1332-1338.

Moody John and R. Lindstrom. Selection and Cleaning of Plastic Containers for Storage of Trace Element Samples. Analytical Chemistry 49, 14 (1977) 2264-2267.

Nakahara H., Y. Nagame, Y. Yoshizawa, H. Oda, S. Gotoh and Y. Murakami. Trace Element Analysis of Human Blood Serum by Neutron Activation Analysis. Journal of Radioanalytical Chemistry 54, 1-2 (1979) 183-190.

Nyarku S.K. and A. Chatt. Removal of Sodium and Chlorine from Seawater prior to Neutron Activation Analysis for Trace Elements. Journal of Radioanalytical Chemistry 71, 1-2 (1982) 129-145.

Olatunbosun D.A., J. O. Bolodeoku, T. O. Cole and B. K. Adadevoh. Relationship of Serum Copper and Zinc to Human Hypertension in Nigerians. Bull. WHO 53 (1976) 134-135.

Olson O.E.. Methods of Analysis for Selenium: a Review. Proceedings of a Symposium: Selenium and Tellurium in the Environment. Pittsburgh, Pa: Industrial Health Foundation (1976) 67-84.

Prasad Ananda S.. Trace Elements in Human Health and Disease. Volume I - Zinc and Copper; Volume II - Essential and Toxic Elements. New York: Academic Press (1976).

Ralston H.R. and E. S. Sato. Sodium Removal as an Aid to Neutron Activation Analysis. Analytical Chemistry 43, 1 (1971) 129-131.

Schmidt G.M., K. G. Blume, K. J. Bross, W. E. Spruce, J. C. Waldron and H. Levine. Parenteral Nutrition in Bone Marrow Transplant Patients. Exp. Hemat. 8, 4 (1980) 506-511.

Speecke A., J. Hoste, J. Versieck. Sampling of Biological Materials. In: Accuracy in Trace Analysis: Sampling, Sample Handling, Analysis. P. D. La Fleur, Ed., NBS Special Publication 422, Vol I, Government Printing Office, Washington (1976) 127-139.

Sullivan J.F., A. J. Blotcky, M. J. Jetton, H. K. J. Hahn. and E. E. Burch. Serum Levels of Selenium, Ca, Cu, Mg, Mn and Zn in Various Human Diseases. Journal of Nutrition 109, 8 (August 1979) 1432-1437.

Torok G., R. Schelenz, E. Fischer and J. F. Diehl. Abtrennung von Na, K, und P mit Hilfe anorganischer Separatoren bei der Neutronenaktivierungsanalyse von biologischem Material. Zeitschrift fur Analytische Chemie 263 (1973) 110-115.

Underwood E.J.. Trace Elements in Human and Animal Nutrition. Third Edition. New York and London: Academic Press, Inc. (1971).

Jacques Versieck and Rita Cornelis. Normal Levels of Trace Elements in Human Blood Plasma or Serum. Analytica Chimica, Acta 116, 2 (1980) 217-254.

Versieck J., J. De Rudder, J. Hoste and F. Barbier. Extraneous Additions During Sampling for Trace Elements Analysis and Results of Determinations in Serum of Healthy Subjects. Trace Substances in Environmental Health XVI A symposium. D. D. Hemphill, Ed., University of Missouri, Columbia (1980) 315-328.

Versieck J., J. Hoste, F. Barbier, H. Michels and J. De Rudder. Simultaneous Determinations of Iron, Zinc, Selenium, Rubidium, and Cesium in Serum and Packed Blood Cells by Neutron Activation Analysis. Clinical Chemistry 23, 10 (1977) 1301-1305.

Versieck J., J. Hoste, F. Barbier, H. Steyaert, J. De Rudder and H. Michels. Determination of Chromium and Cobalt in Human Serum by Neutron Activation Analysis. Clinical Chemistry 24, 2 (1978) 303-308.

Versieck J., J. Hoste, L. Vanballenberghe , F. Barbier, R. Cornelis and I. Waelput. Serum Molybdenum in Diseases of the Liver and Biliary System. The Journal of Laboratory and Clinical Medicine 97, 4 (1981) 535-544.

Versieck J. and A. Speecke. Contaminations Induced by Collection of Liver Biopses and Human Blood. Nuclear Activation in the Life Sciences A symposium held in Bled, Yugoslavia by the International Atomic Energy Agency, Viena (1972) 39-49.

Versieck J., A. Speecke, J. Hoste and F. Barbier. Determination of Manganese, Copper and Zinc in Serum and Packed Blood Cells by Neutron Activation Analysis. Z. Klin. Chem. Klin. Biochem. 11 (1973) 193-196.

Ward Neil I., Roger Stephens and Douglas E. Ryan. Comparison of Three Analytical Methods for the Determination of Trace Elements in Whole Blood. Analytica Chimica, Acta, 110, 1 (1979) 9-19.

Webster's New Collegiate Dictionary, G. & C. Merriam Company, Springfield, Massachusetts (1976).

Woittiez J. R. W. and H. A. Das. On the Possibilities of Elemental Analysis of Human Serum by Neutron Activation Analysis to Short Lived Radionuclides with Previous Removal of Sodium and Chlorine. Journal of Radioanalytical Chemistry 68, 1-2 (1982) 67-73.

## BIOGRAPHICAL SKETCH

Eduardo Mussnica Barreto was born January 14, 1956, in Bento Goncalves, RS, Brazil. The son of an Army officer, he attended secondary and high school at military establishments.

In 1978, he graduated with a bachelor's degree in electronic engineering at the Federal University of Rio Grande do Sul, Brazil. After graduation he applied to attend graduate studies toward a master's degree in nuclear engineering at the Military Institute of Engineering in Rio de Janeiro, under the sponsorship of the Brazilian Commission for Nuclear Energy. A monthly scholarship was also awarded, as part of that Agency's sponsorship.

A year later, he was invited to extend his graduate studies toward a doctor's degree in a foreign country, in any field related to the nuclear science.

After 2 years of studies at the University of Florida he returned on vacation to meet his 1 year old sister Andrea, and ended up also meeting Carmen who is now his wife.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Genevieve L. Roessler

Genevieve Roessler, Ph.D., Chairman
Associate Professor of
Environmental Engineering Sciences
and Nuclear Engineering Sciences

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

W. Emmett Bolch

W. E. Bolch, Jr, Ph.D., Cochairman
Professor of Environmental
Engineering Sciences

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

John A Wethington Jr.

John Wethington, Jr, Ph.D.
Professor of Nuclear Engineering
Sciences

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Phillip Achey

Phillip Achey, Ph.D.
Professor of Microbiology and Cell
Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Joseph Delfino, Ph.D.
Professor of Environmental
Engineering Sciences

This dissertation was submitted to the Graduate Faculty of the College of Engineering and to the Graduate School, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August 1984

Hubert A. Bevis
Dean, College of Engineering

Dean for Graduate Studies and
Research